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(57) Abstract

2-Thioindoles (2-selenoindoles) and analogous 2-indolinethione (2-indolineselenone) and polysulfide (selenide) compounds, salts thereof, methods of production, intermediates in their production, pharmaceutical compositions containing said compounds, and methods for inhibiting protein kinase dependent disease in a mammal or treating aberrant cell growth in a mammal, using said compositions, are disclosed.

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2-THIOINDOLES (SELENOINDOLES) AND RELATED DISULFIDES (SELENIDES) WHICH INHIBIT PROTEIN TYROSINE KINASES AND WHICH HAVE ANTITUMOR PROPERTIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of co-pending application U.S. Serial Number 926,015, filed August 6, 1992.

FIELD OF INVENTION

The present invention relates to substituted 2-thioindoles (selenoindoles) and other related compounds, which we have unexpectedly found to be potent inhibitors of the epidermal growth factor receptor tyrosine kinase (EGF-TK) and other protein tyrosine kinases, and which show antitumor activity. The invention also relates to use of the compounds as inhibitors of protein tyrosine kinases and as antitumor agents.

BACKGROUND OF THE INVENTION

Protein phosphorylation is a critical mechanism for regulating protein function in the signal transduction pathway in normal and transformed cells. Protein tyrosine kinases (PTK) are an important class of phosphorylating enzymes which mediate this signalling and thereby regulate cell growth and proliferation. PTKs catalyze the transfer of the terminal phosphate from ATP to the phenol of tyrosine

protooncogenes and oncogene products possess PTK activity. The overexpression or inappropriate expression of normal or mutant kinases can result in the loss of growth control and the unregulated cell proliferation associated with malignancy. Small molecules which selectively inhibit these enzymes are, therefore, of therapeutic interest as mediators of cell growth and as antitumor agents.

In some growth factor dependent tumors, the growth factor signal transduction pathway employs the intrinsic tyrosine kinase activity of the growth factor receptor for autophosphorylation and the phosphorylation of specific cellular proteins involved in mitogenesis and cell proliferation. Specific inhibitors of PTKs have been identified previously. It has been previously demonstrated that by uncoupling the PTK from the signal transduction pathway, inhibitors of the growth factor receptor tyrosine kinases result therapeutically in antitumor activity. This antitumor activity has been demonstrated both in vitro and in vivo. Most known tyrosine kinase inhibitors are styrene-like small molecules in which the aromatic ring is hydroxylated, resembling tyrosine itself.

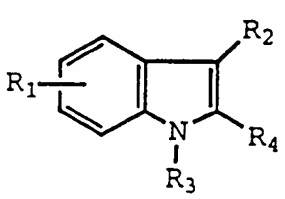
For example, the EGF-TK inhibitor erbstatin is reported to inhibit the growth of human epidermoid carcinoma A431 cells with an IC_{50} = 3.6 μg/mL (J. Antibiot. 1986;39:170). Erbstatin also inhibits the growth of the human mammary carcinoma MCF-7 and some esophageal tumors in nude mice in a dose-dependent manner (Eur. J. Cancer 1990;26(6):722 and Japanese Patent 03,109,323). Another class of PTK inhibitor called the tyrphostins also potently inhibited the EGF-dependent growth of A431 cells in vitro (J. Med. Chem. 1989;32:2344; J. Med. Chem. 1991;34:1896). The antitumor activity of two tyrphostins has been verified

carcinoma MH-85 (Cancer Res. 1991;51:4430). In vitro and in vivo antitumor activity against A431 tumors has also been reported for a series of sulfonylbenzoyl nitrostyrenes (J. Med. Chem. 1991;34:2328) as TK inhibitors (J. Med. Chem. 1991;34:2328 and Helv. Chim. Acta 1992;75:696).

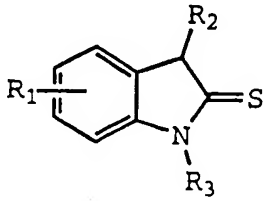
SUMMARY AND DETAILED DESCRIPTION

In one aspect, the invention relates to 2-thioindole (selenoindoles) and other related compounds that are potent inhibitors of epidermal growth factor receptor tyrosine kinase and other protein tyrosine kinases, and which have antitumor activity. Thus, the compounds are useful in dosage form as inhibitors of protein tyrosine kinases and as antitumor agents.

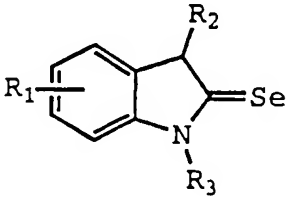
More particularly, the invention comprises 2-thioindole, 2-indolinethione, polysulfide, 2-selenoindole, 2-indolineselenone, and selenide compounds represented by the general Formulas I, IV, and XXXII



I



IV



XXXII

and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OCOR, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and

R_2 is a member selected from

C_{2-4} alkyl,

$(CH_2)_nCOOH$,

$(CH_2)_nCOOR$,

$(CH_2)_nCOR$,

$(CH_2)_nSO_2R$,

$(CH_2)_nSO_2NRR$,

$(CH_2)_nSO_2NHR$,

$CH{=}CHCOOH$,

$(CH_2)_nCH(OH)\text{-}COOH$,

$(CH_2)_nCH(NH_2)\text{-}COOH$,

$(CH_2)_nCONH_2$,

$(CH_2)_nCONHR$,

$(CH_2)_nCONRR$,

$(CH_2)_nCONHCH_2Ph$,

CONHR,

CONRR,

CONHPh,

COY,

COPhCOOH,

COPhCOOR,

$(CH_2)_nCONHPh$,

$(CH_2)_nCONHPhR$,

SO_2Y;

n is an integer from 1 to 4;

R is lower alkyl, preferably C_{1-4} alkyl;

R_3 is a member selected from H, lower alkyl, and benzyl;

Y represents a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

lower alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-thioindolyl or 2-selenoindolyl moiety of Formula I provided that the group does not comprise compounds having the names

2-(2-thioxo-3-indolinyl)acetic acid,
2-(1-methyl-2-thioxo-3-indolinyl)acetic acid,
methyl 2-(2-thioxo-3-indolinyl)acetate,
ethyl 2-(1-methyl-2-thioxo-3-indolinyl)acetate,
bis[methylindolinyl-3-acetate-(2)]disulfide,
bis[indolyl-3-acetic acid-(2)]disulfide,
bis[methylindolyl-3-acetate-(2)]trisulfide, and
bis[1-methylindolyl-3-acetic acid-(2)]disulfide.

In another aspect, the invention relates to indolinethione compounds of the above Formula IV which exist as tautomers of compounds of Formula I wherein R_4 represents SH or indolineselenone compounds of the above Formula XXXII which exist as tautomers of compounds of Formula I wherein R_4 represents SeH. The invention comprises the thione or selenone compounds in their racemic and optical isomer forms. The thione or selenone compounds produced in the (±) form can be resolved as their (+) and (-) enantiomorphic optical isomers by per se art-recognized conventional means such as fractional crystallization of salts formed from optically active acids, separation of the isomers by chiral chromatography, or the chiral catalytic reduction of precursors.

In another aspect, the invention relates to pharmaceutical compositions useful for inhibition of protein tyrosine kinases and for antitumor activity containing as an active agent in a pharmaceutically acceptable carrier a therapeutically effective amount of a compound selected from 2-thioindole,

2-indolineselenone or selenide compounds represented by the above Formulas I, IV, and XXXII and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OCOR, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and further represents replacement in the ring of 1 or 2 ring methine (-CH=) atoms with aza(-N=) atoms;

R_2 is a member selected from

lower alkyl, preferably C_{1-4} alkyl,

$(CH_2)_nCOOH$,

$(CH_2)_nCOOR$,

$(CH_2)_nCOR$,

$(CH_2)_nSO_2R$,

$(CH_2)_nSO_2NRR$,

$(CH_2)_nSO_2NHR$,

CH=CHCOOH,

$(CH_2)_nCH(OH)COOH$,

$(CH_2)_nCH(NH_2)COOH$,

$(CH_2)_nCONH_2$,

$(CH_2)_nCONHR$,

$(CH_2)_nCONRR$,

$(CH_2)_nCONHCH_2Ph$,

CONHR,

CONRR,

CONHPh,

COY,

COPhCOOH,

COPhCOOR,

$(CH_2)_nCONHPh$,

$(CH_2)_nCONHPhR$,

SO_2Y;

n is an integer from 1 to 4;

R_3 is a member selected from H, lower alkyl and benzyl;

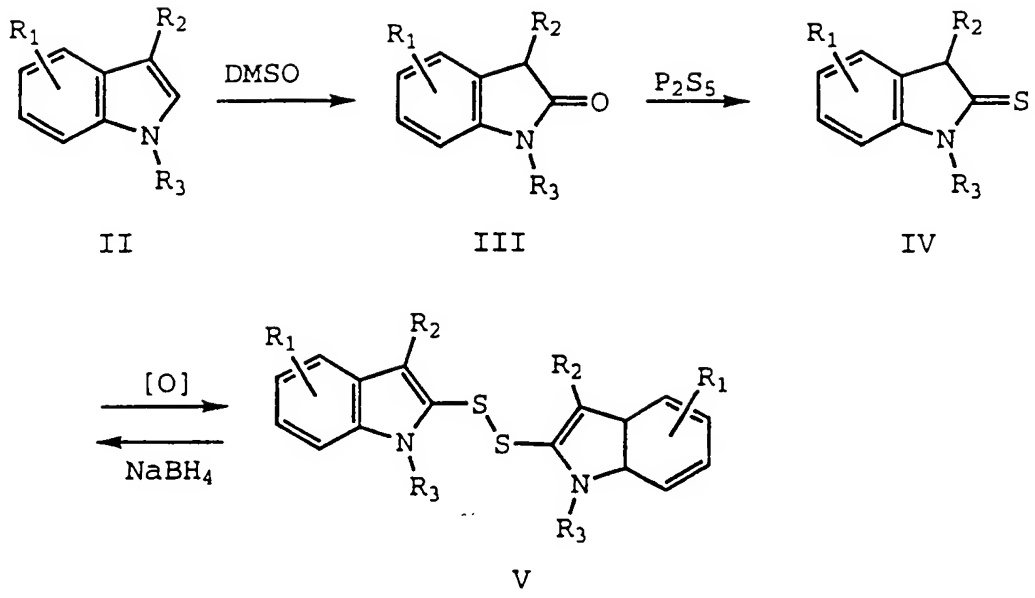
Y represents a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

R_4 represents SH, S_oX, S_oQ, SeH, Se_oX, and Se_oQ, where o is 1, 2, or 3, X is a member selected from H, lower alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-thioindolyl or 2-selenoindolyl moiety of Formula I.

The invention comprises salt compounds formed by the basic or acidic thioindole compounds of the invention which form pharmaceutically acceptable salts with both organic and inorganic acids and/or organic and inorganic bases. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic, and the like. Examples of suitable bases for salt formation are sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine, and the like.

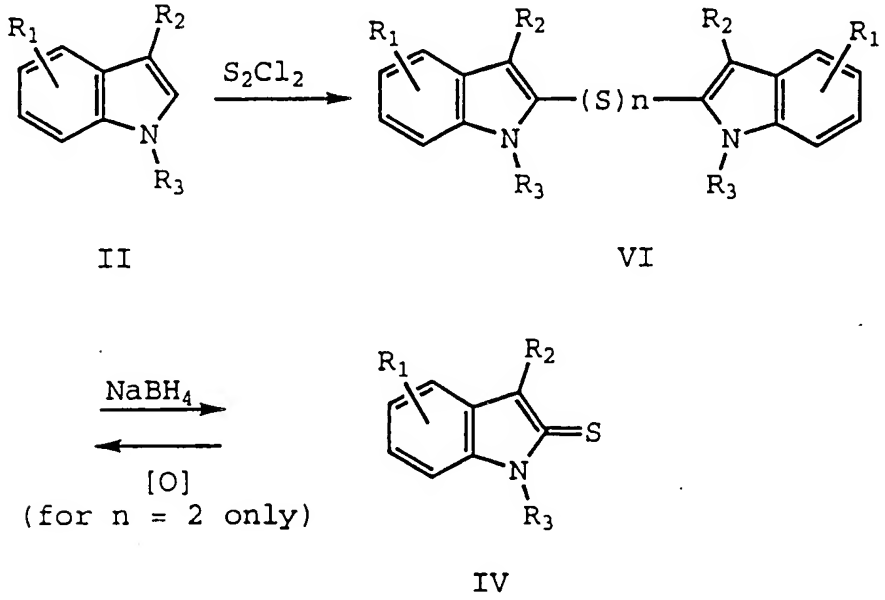
The compounds of Formulas I, IV, and XXXII can be prepared by the processes described in the following Reaction Schemes 1-11.

SCHEME 1



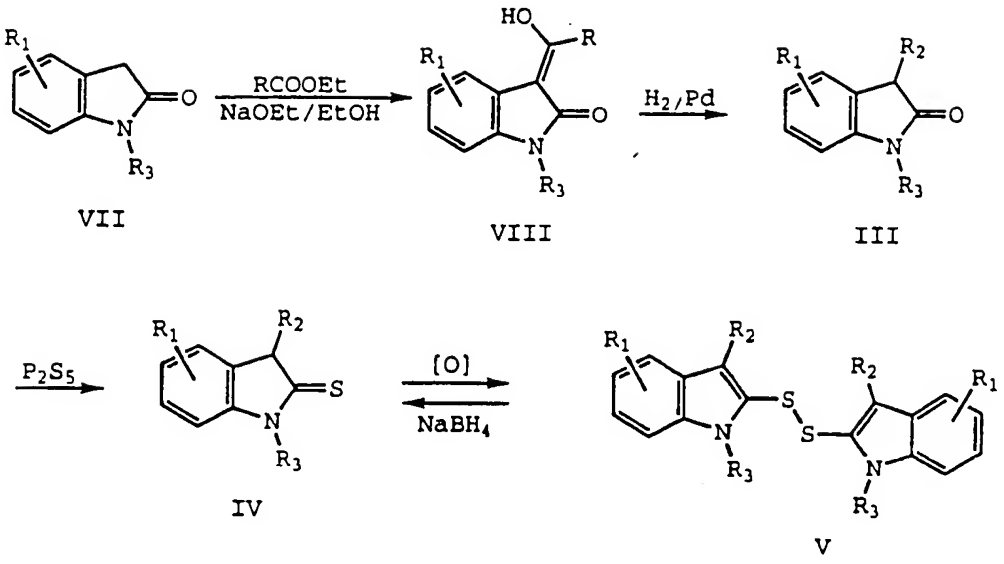
In Scheme 1, R_1-R_3 are as designated for Formula I. Oxidation of 3-substituted indoles **II** in DMSO/HCl gives good yields of 3-substituted indolin-2-ones **III** which are thiated (preferably with P_2S_5 and $NaHCO_3$ or Na_2CO_3) to yield 3-substituted 2-indolinethiones **IV**. These compounds can be converted to the corresponding disulfides **V** by treatment with mild oxidizing agents (e.g., $FeCl_3$), and also undergo spontaneous oxidation to **V** in solution in air.

SCHEME 2



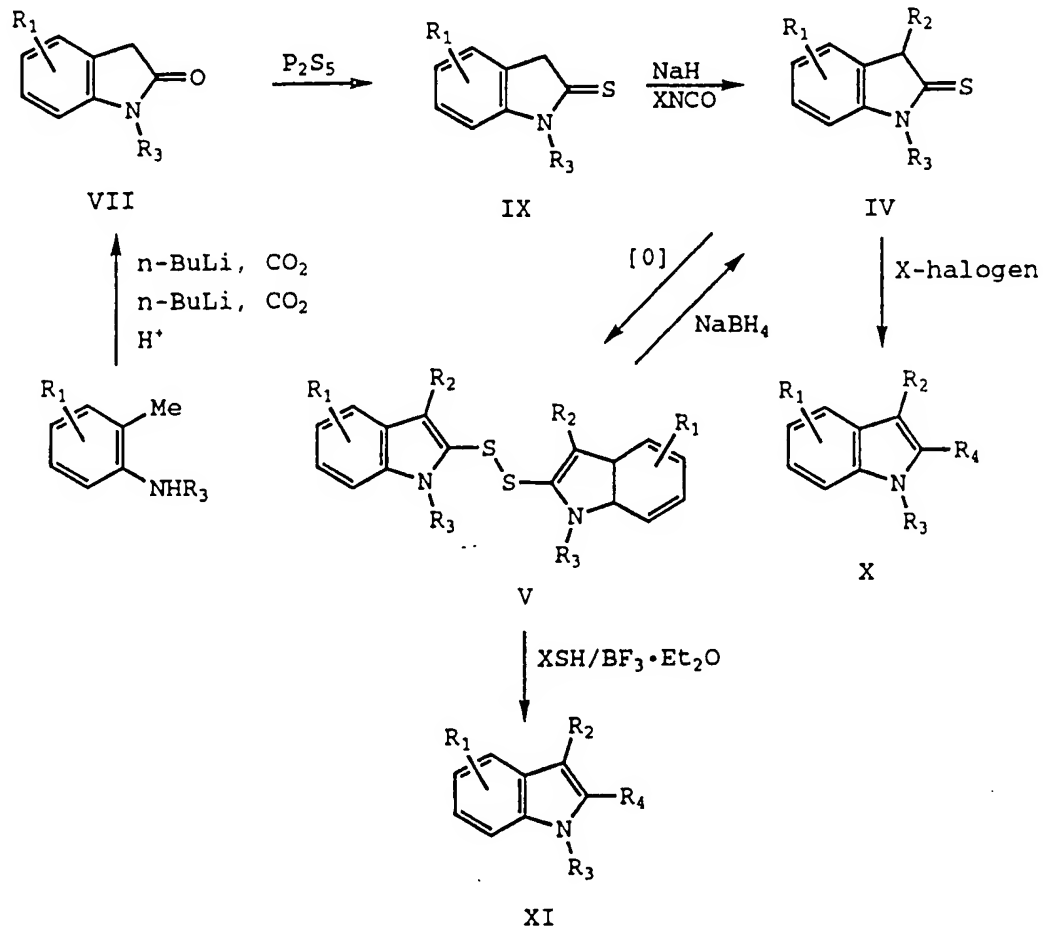
In Scheme 2, R_1-R_3 are as designated for Formula I. Treatment of 3-substituted indoles **II** with S_2Cl_2 gives mixtures of dimeric sulfides **VI**, where n = 1-3. These can be separated by chromatography, or more conveniently reduced to 2-indolinethiones **IV** with a mild reducing agent (preferably $NaBH_4$).

SCHEME 3



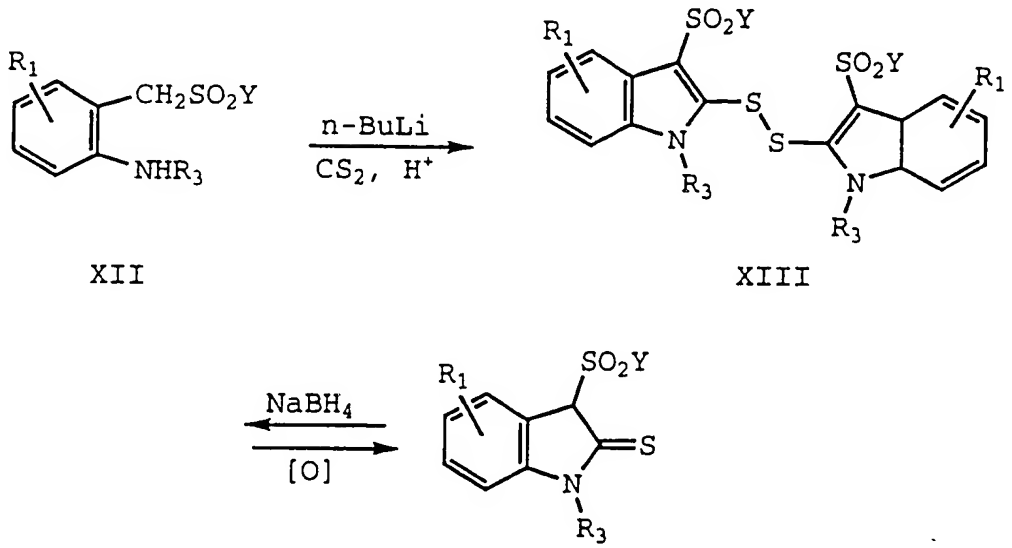
In Scheme 3, R_1-R_3 are as designated for Formula I, and R represents $(CH_2)_nCOOH$, $(CH_2)_nCOOX$, $(CH_2)_nCONHX$, $(CH_2)_nSO_2X$, or $(CH_2)_nSO_2NX$, where n is from 0 to 4, and X is as designated for Formula I. Treatment of 2-indolinones **VII** with diesters gives moderate yields of the isatylidene compounds **VIII**, which can be hydrogenated under acidic conditions to the 3-substituted indolin-2-ones **III**. Treatment of these as in Scheme 1 gives the desired compounds.

SCHEME 4


R1
O
N
R3
VII
P2S5
S
IX
NaH
XNCO
R2
IV
n-BuLi, CO2
n-BuLi, CO2
H+
[0]
NaBH4
X-halogen
Me
NHR3
S
V
R4
X
XSH/BF3•Et2O
XI

In Scheme 4, R_1-R_4, R and X are as designated for Formula I (except that X is not H). The ring-substituted oxindoles can be prepared by lithiation of the appropriately substituted ortho-toluidine derivatives, using CO_2 as both the N-protecting group and electrophile (Katritzky, Fan, Akutagawa, Wang, Heterocycles 1990;30:407). 2-Indolinones **VII** are thiated (preferably with P_2S_5 and $NaHCO_3$ or Na_2CO_3) to yield 2-indolinethiones **IX**. These compounds are deprotonated (typically with NaH in THF), and treated with an isocyanate to give 3-substituted 2-indolinethiones **IV** (where R_2 = CONHX). These compounds can be converted to the corresponding disulfides **V** as described in Scheme 1. The 3-substituted 2-indolinethiones **IV** can also react with alkylating agents (typically alkyl halides R-halogen) to give (**X**: where R_4 = X). Reaction of **V** with XSH gives mixed disulfides (**XI**: where R_4 = SSX).

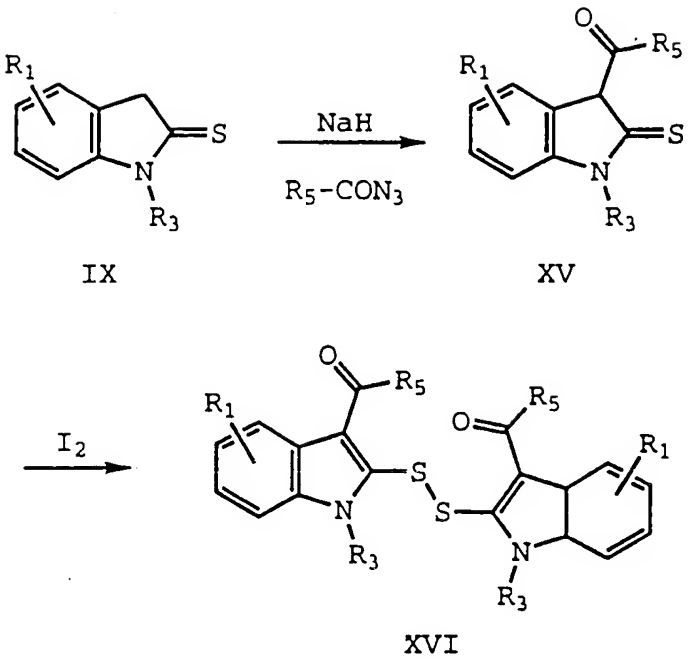
SCHEME 5



XII XIII XIV

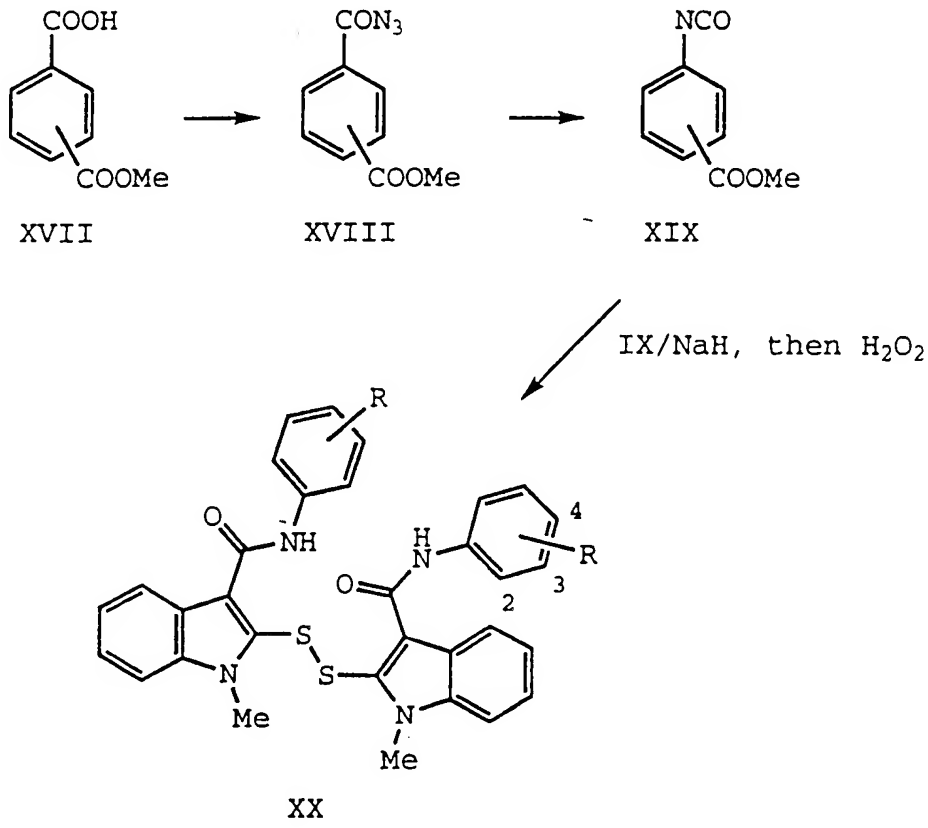
In Scheme 5, R_1 and R_3 are as designated for Formula I and Y represents lower alkyl or a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring, optionally substituted with a lower alkyl, COOH, OH, NH_2, CONHR, OR, O, or NHR group. 2-Sulfonylmethyl anilines **XII** are treated sequentially with n-butyllithium and CS_2, to give the disulfides **XIII**, which can be reduced to 2-indolinethiones **XIV** with a mild reducing agent (preferably $NaBH_4$).

SCHEME 6



In Scheme 6, R_1 and R_3 are as designated for Formula I. Deprotonation of substituted 2-indolinethiones **IX** (typically with NaH in THF), followed by treatment with an acyl azide, gives 3-acyl-substituted 2-indolinethiones **XV**, where R_5 represents H, lower alkyl, benzyl, or a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a COOH, OH, NH_2, CONHR, OR, NHR, or NRR group. Compounds **XV** can be converted into the disulfides **XVI** on mild oxidation (typically by treatment with I_2 or H_2O_2).

SCHEME 7

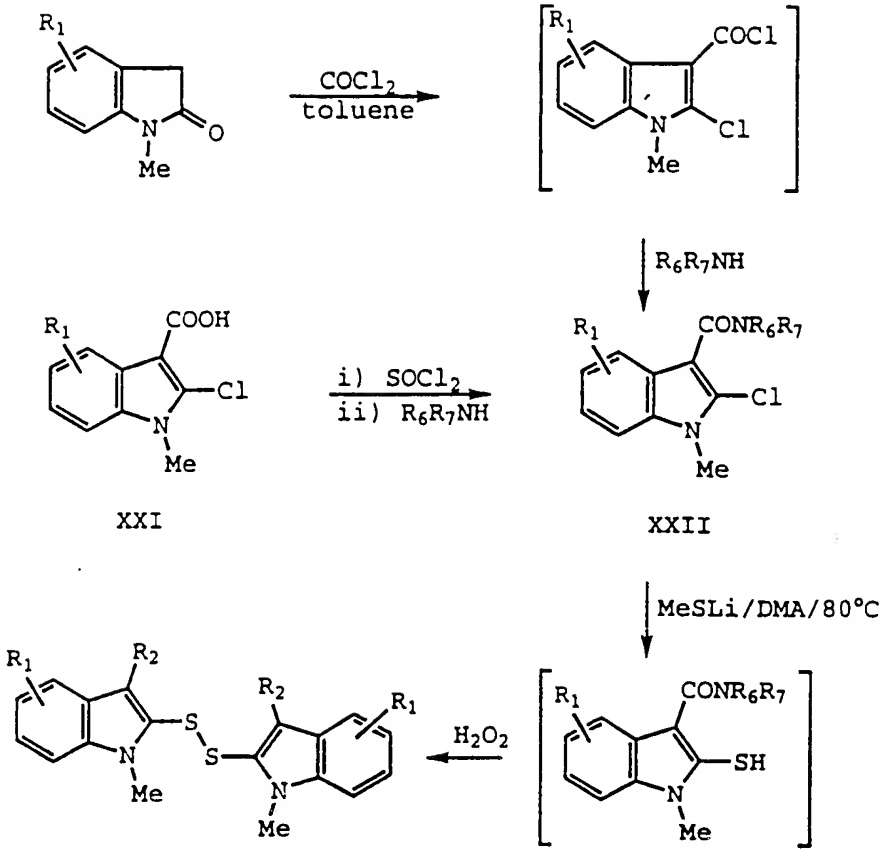


XVII XVIII XIX

XX

In Scheme 7, R is as designated for Formula I. Substituted aromatic and heteroaromatic acids (e.g., XVII) are converted to the corresponding acid chlorides (preferably with $SOCl_2$), and then to the corresponding acyl azides (e.g., XVIII) with NaN_3. Rearrangement to give the isocyanates (e.g., XIX) is carried out in an inert solvent (preferably toluene or xylene). These isocyanates (e.g., XIX) are converted to the disulfides (XX) by reaction with the sodium salt of 1-methyl-2-indolinethiones as outlined in Scheme 4. In suitable cases, hydrolysis of esters (XX; R = COOMe) with a mild base (preferably K_2CO_3) gives the corresponding acids

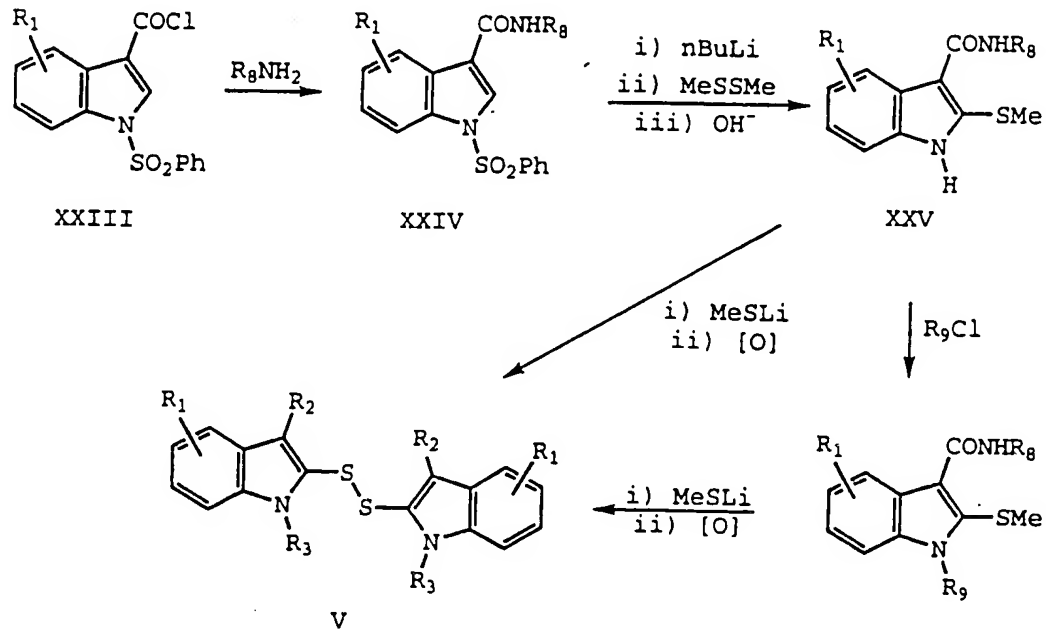
SCHEME 8



XXI XXII V

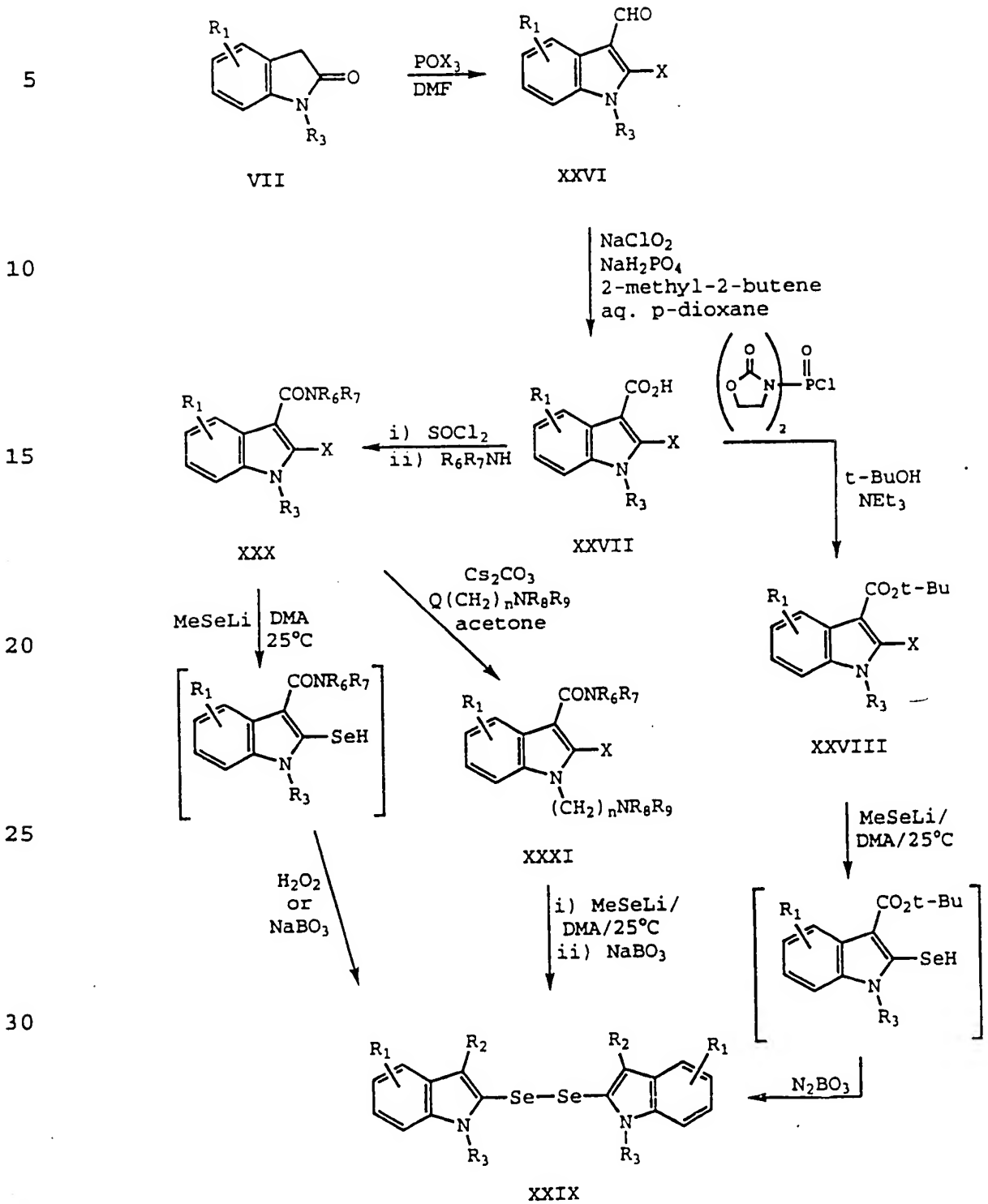
In Scheme 8, R_1 and R_2 are as designated for Formula I, and R_6 and R_7 are individually H, lower alkyl, benzyl, or a benzene ring optionally substituted with up to two of the groups COOH, OH, NH_2, CONHR, OR, NHR, or NRR. 2-Chloro-1-methylindole-3-carbonyl chloride, prepared either from indolin-2-one and $COCl_2$ or from 2-chloro-1-methylindole-3-carboxylic acid (XXI) and $SOCl_2$, is reacted with amines HNR_6R_7 or their salts, in an inert solvent (preferably 1,2-dichloroethane or CH_2Cl_2) and a base, if necessary, to give the amides (XXII). These compounds are heated with MeSLi in polar aprotic solvents (preferably dimethylacetamide) in an inert atmosphere to give intermediate thiol carboxamides, which are oxidized, (preferably with H_2O_2) to give the desired disulfides (V).

SCHEME 9



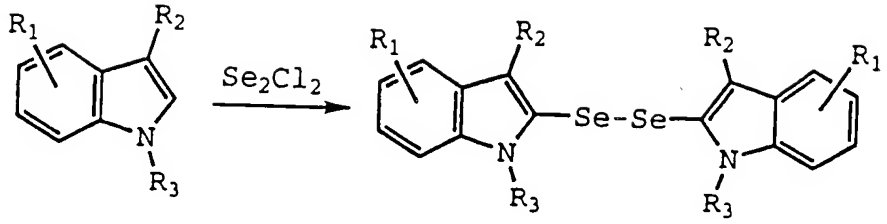
In Scheme 9, R_1, R_2, R_3, and R are as designated for Formula I. Reaction of acid chloride (XXIII) with amines gives amides (XXIV), where R_8 represents H, lower alkyl, benzyl, or a benzene ring optionally substituted with up to two of the groups COOH, OH, NH_2, CONHR, OR, NHR, or NRR. Compounds (XXIV) can be converted to 2-thioindoles (XXV) by lithiation and quenching with methyl sulfide, followed by base hydrolysis (preferably with K_2CO_3). The 2-thioindoles (XXV) can be converted to the desired disulfides (V) by dealkylation (preferably with lithium thiomethoxide) and mild oxidation (preferably with I_2 or H_2O_2). Compounds (XXV) can also be alkylated with an alkyl halide (e.g., R_9Cl), where R_9 represents lower alkyl, benzyl, or benzyl optionally substituted with up to two of the groups COOH, OH, NH_2, CONHR, OR, NHR, or NRR,

SCHEME 10


R1
O
N
R3
POX3
DMF
CHO
X
VII
XXVI
NaClO2
NaH2PO4
2-methyl-2-butene
aq. p-dioxane
CONR6R7
CO2H
i) SOCl2
ii) R6R7NH
PCl
t-BuOH
NEt3
XXX
XXVII
Cs2CO3
Q(CH2)nNR8R9
acetone
MeSeLi
DMA
25°C
CO2t-Bu
XXVIII
SeH
(CH2)nNR8R9
XXXI
MeSeLi/
DMA/25°C
H2O2
or
NaBO3
i) MeSeLi/
DMA/25°C
ii) NaBO3
R2
Se
N2BO3
XXIX

In Scheme 10, R_1 is as designated for Formula I and R_6 and R_7 are individually H_1 lower alkyl, benzyl, or a benzene ring optionally substituted with up to two of the groups COOH, OH, NH_2, CONHR, OR, NHR, or NRR. R_3 is H or lower alkyl, and X = any halogen, preferably bromine or chlorine. Substituted 2-halo-3-indole carboxylic acids XXVII, prepared by oxidation of corresponding substituted 3-carboxaldehydes, are reacted with amines HNR_6R_7 or their salts in an inert solvent (preferably 1,2-dichloroethane or CH_2Cl_2) and a base, if necessary, to give the amides XXX. These compounds are reacted with MeSeLi in polar aprotic solvents (preferably dimethylacetamide) to give intermediate selenol carboxamides, which are oxidized with H_2O_2 or $NaBO_4$ to give the desired diselenides XXIX. Alternatively, intermediate XXX, where R_3 = H, can be reacted with a haloalkyl amine, or its salt, where Q = Cl, Br, I (preferably Cl) and R_8, R_9 are as defined in Formula I, but preferably R_8 and R_9 are H, alkyl, cycloalkyl, and n = 1-4 in a polar solvent (preferably acetone) and anhydrous metal carbonate (preferably cesium carbonate) to give intermediate XXXI which is converted to diselenide XXIX as described above for intermediate XXX. Additionally, intermediate acid XXVII can be converted to the substituted 2-halo-3-indole carboxylic acid tertiary butyl ester XXVIII, which can be further reacted with MeSeLi as described above for intermediate XXX to give the target substituted diselenide XXIX where R_2 = COO-tertiarybutyl.

SCHEME 11



II XXIX

In Scheme 11, R_1-R_3 are as designated for Formula I. Treatment of 3-substituted indoles II with Se_2Cl_2 gives the diselenide XXIX.

As indicated, the compounds of this invention that are basic can form acidic salts and those that are acidic can form basic salts. All such salts are within the scope of this invention and they can be prepared by conventional methods. For example, they can be prepared simply be contacting the acidic and basic entities, usually in a stoichiometric ratio, in either an aqueous, nonaqueous or partially aqueous medium, as appropriate. The salts are recovered either by filtration, by precipitation followed by filtration, by evaporation of the solvent, or in the case of aqueous solutions, by lyophilization, as appropriate.

The compounds of this invention are readily adapted to therapeutic use for the control of tyrosine kinase dependent diseases in mammals. Tyrosine kinase dependent diseases comprise hyperproliferative disorders which are initiated and/or maintained by aberrant tyrosine kinase enzyme activity. Tyrosine kinase inhibitors can therefore have beneficial therapeutic effects against aberrant cell growth disorders such as various cancers, atherosclerosis, angiogenesis (tumor growth/metastasis, diabetic retinopathy, for example), viral diseases (HIV infections, for example), and the like.

Tyrosine kinase dependent diseases further comprise cardiovascular diseases which are related to aberrant tyrosine kinase enzyme activity. Tyrosine kinase inhibitors can therefore have beneficial therapeutic effects against such cardiovascular diseases as restenosis. It should be understood that restenosis is an example of a cardiovascular disease which is dependent upon tyrosine kinase; one skilled in the art, however, will be aware of other examples of cardiovascular diseases which are dependent upon tyrosine kinase.

The compounds are administered either orally or parenterally, or topically as eye drops, in dosages ranging from about 0.1 to 10 mg/kg of body weight per day in single or divided doses. Of course, in particular situations, at the discretion of the attending physician, doses outside of this range will be used.

The compounds of this invention can be administered in a side variety of different dosage forms, i.e., they may be combined with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, elixirs, syrups, injectable or eye drop solution, and the like. Such carriers include solid diluents or fillers, sterile aqueous media, and various nontoxic organic solvents.

For purposes of oral administration, tablets containing various excipients such as sodium citrate, calcium carbonate, and calcium phosphate are employed along with various disintegrants such as starch and preferably potato or tapioca starch, alginic acid, and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin, and acacia. Additionally, lubrication agents such as magnesium stearate, sodium lauryl sulfate, and talc are often very useful for tableting purposes. Solid compositions of similar type are also employed as fillers in soft- and hard-filled gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the essential active ingredient therein can be combined with various sweetening agents, flavoring agents, coloring agents, emulsifying agents, and/or suspending

propylene glycol, glycerin, and various like combinations thereof.

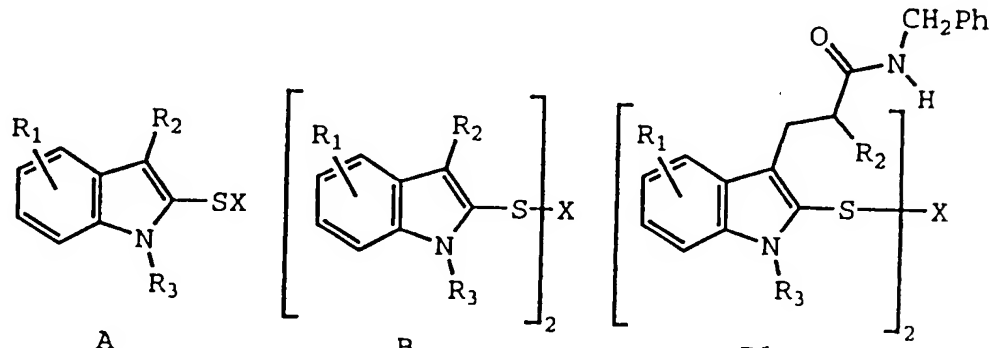
For purposes of parenteral administration, solutions in sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions of the corresponding water soluble, alkali metal, or alkaline earth metal salts previously enumerated. Such aqueous solution should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well known to those skilled in the art.

For purposes of topical administration, dilute sterile, aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared in containers suitable for dropwise administration to the eye.

In a pharmaceutical composition comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, the weight ratio of carrier to active ingredient will normally be in the range from 1:4 to 4:1, and preferably 1:2 to 2:1. However, in any given case, the ratio chosen will depend on such factors as the solubility of the active component, the dosage contemplated and the precise route of administration.

The following Table 1 sets out physical data for 137 compounds within the general Formula I, representative of it, and preparable by the processes of the invention.

TABLE 1



A B B1

.	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
1	A	H	CH_2COOH	H	H	166-168	$C_{10}H_9NO_2S$	known[d]
2	A	H	CH_2COOH	Me	H	150-153	$C_{11}H_{11}NO_2S$	known[d]
3	A	H	CH_2COOMe	H	H	150-152	$C_{11}H_{11}NO_2S$	C,H,N,S[e]
4	A	H	CH_2COOMe	Me	H	68-70	$C_{12}H_{13}NO_2S$	C,H,N,S
5	A	H	CH_2COOEt	Me	H	47-48	$C_{13}H_{15}NO_2S$	C,H,N,S[e]
6	A	H	$CH_2CONHCH_2Ph$	H	H	193-195	$C_{17}H_{10}N_2OS$	C,H,N,S
7	A	H	$(CH_2)_2COOH$	H	H	170-173	$C_{11}H_{11}NO_2S$	C,H,N
8	A	H	$(CH_2)_2COOH$	Me	H	126-128.5	$C_{12}H_{13}NO_2S \cdot 0.25H_2O$	C,H,N,S
9	A	H	$(CH_2)_2COOMe$	H	H	95.5-98	$C_{12}H_{13}NO_2S$	C,H,N,S

TABLE 1 (cont'd)

.	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
10	A	H	$(CH_2)_2COOEt$	H	H	oil[b]	$C_{13}H_{15}NO_2S$	C,H,N,S
11	A	H	$(CH_2)_2COOMe$	Me	H	71-73	$C_{13}H_{15}NO_2S$	C,H,N,S
12	A	H	$(CH_2)_2COOEt$	Me	H	61-63	$C_{14}H_{17}NO_2S$	C,H,N,S
13	A	H	$(CH_2)_2CONHCH_2Ph$	H	H	149.5-151	$C_{18}H_{18}NO_2S \cdot 0.5H_2O$	C,H,N,S
14	A	H	$(CH_2)_2CONH_2$	H	H	160-163	$C_{11}H_{12}N_2OS$	C,H,N,S
15	A	H	$(CH_2)_3COOH$	H	H	132-134	$C_{12}H_{13}NO_2S$	C,H,N,S
16	A	H	$(CH_2)_3COOH$	Me	H	144-146.5	$C_{13}H_{15}NO_2S \cdot H_2O$	C,H,N,S
17	A	H	$(CH_2)_3COOMe$	H	H	109-110	$C_{13}H_{15}NO_2S$	C,H,N,S
18	A	H	$(CH_2)_3COOMe$	Me	H	103-106	$C_{14}H_{17}NO_2S$	C,H,N,S
19	A	7-aza	CONHPh	Me	H	162-164	$C_{15}H_{13}N_3O_2S \cdot CH_3OH$	C,H,N,S
20	A	5-Cl	CONHPh	Me	H	312-320	$C_{16}H_{13}ClN_2OS$	HRMS
21	A	H	CONHPh	Me	H	149-151	$C_{16}H_{14}N_2OS$	C,H,N,S
22	A	H	CONHPh	Me	Me	116-118	$C_{17}H_{16}N_2OS$	C,H,N,S
23	A	H	CONHPh	Me	CH_2Ph	144-146	$C_{23}H_{20}N_2OS_2$	C,H,N,S
24	A	H	COPh	Me	H	130-132	$C_{16}H_{13}NOS$	C,H,N,S
25	A	H	COPhpCOOH	Me	H	282 (dec)	$C_{17}H_{13}NO_3S \cdot 0.25H_2O$	C,H,N
26	A	H	COPhpCOOMe	Me	H	164-166	$C_{18}H_{15}NO_3S$	C,H,N,S
27	B	H	CH_2COOMe	H	-	160-162	$C_{22}H_{20}N_2O_4S_2$	C,H,N,S[f]
28	B	H	CH_2COOMe	Me	-	130-132.5	$C_{24}H_{24}N_2O_4S_2$	C,H,N,S
29	B	H	CH_2COOH	H	-	196-199	$C_{20}H_{16}N_2O_4S_2$	known[d]
30	B	H	CH_2COOH	H	S	199-202	$C_{20}H_{16}N_2O_4S_3$	C,H,N,S
31	B	H	CH_2COOMe	H	S	130-132	$C_{22}H_{20}N_2O_4S_3$	C,H,N,S[f]
32	B	H	CH_2COOH	Me	-	190-192.5	$C_{22}H_{20}N_2O_4S_2$	known[d]

TABLE 1 (cont'd)

.	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
3	B	H	CH_2COOEt	Me	-	117-119	$C_{26}H_{28}N_2O_4S_2$	C,H,N,S
4	B	H	$CH_2CONHCH_2Ph$	H	-	200.5-203.5	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
5	B	H	CH_2CN	H	-	168.5-169.5	$C_{20}H_{14}N_4S_2$ (lit ref)[g]	C,H,N,S
6	B	H	$(CH_2)_2COOH$	H	-	118-120.5	$C_{22}H_{20}N_2O_4S_2 \cdot H_2O$	C,H,N,S
7	B	H	$(CH_2)_2COOH$	Me	-	158.5-160	$C_{24}H_{24}N_2O_4S_2$	C,H,N,S
8	B	H	$(CH_2)_2COOEt$	H	-	137-139	$C_{26}H_{28}N_2O_4S_2$	C,H,N,S
9	B	H	$(CH_2)_2COOMe$	H	-	162.5-164	$C_{24}H_{24}N_2O_4S_2$	C,H,N,S
0	B	H	$(CH_2)_2COOMe$	Me	-	139-141.5	$C_{26}H_{28}N_2O_4S_2$	C,H,N,S
1	B	5-Me	$(CH_2)_2COOH$	H	-	91.5-95	$C_{24}H_{24}N_2O_4S_2$	HRMS[c]
2	B	5-Me	$(CH_2)_2COOEt$	H	-	138.5-139	$C_{28}H_{32}N_2O_4S_2 \cdot 0.5C_6H_6$	C,H,N,S
3	B	6-Me	$(CH_2)_2COOH$	H	-	126-128	$C_{24}H_{24}N_2O_4S_2 \cdot 0.5H_2O$	C,H,N,S
4	B	6-Me	$(CH_2)_2COOEt$	H	-	122-123.5	$C_{28}H_{32}N_2O_4S_2$	C,H,N,S
5	B	7-Me	$(CH_2)_2COOH$	H	-	172.5-175	$C_{24}H_{24}N_2O_4S_2$	C,H,N
6	B	7-Me	$(CH_2)_2COOEt$	H	-	120-122.5	$C_{28}H_{32}N_2O_4S_2$	C,H,N,S
7	B	H	$(CH_2)_2CONHCH_2Ph$	H	-	141-144	$C_{36}H_{34}N_4O_2S_2$	C,H,N,S
8	B	H	$(CH_2)_2CN$	H	-	167-169	$C_{21}H_{16}N_4S_2$ (lit ref)[g]	
9	B	H	$(CH_2)_2NO_2$	H	-	153-154	$C_{20}H_{18}N_4O_4S_2 \cdot 0.5H_2O$	C,H,N,S
0	B	H	$(CH_2)_2CONH_2$	H	-	101 (dec)	$C_{22}H_{22}N_4O_2S_2 \cdot 0.5H_2O$	C,H,N,S
1	B	H	$(CH_2)_2CONHMe$	H	-	162.5-164	$C_{24}H_{26}N_4O_2S_2$	C,H,N,S
2	B	H	$(CH_2)_2CONHOMe$	H	-	176-178	$C_{24}H_{26}N_4O_4S_2$	C,H,N,S
3	B	H	$(CH_2)_2CONMe_2$	H	-	179-180	$C_{26}H_{30}N_4O_2S_2$	C,H,N,S
4	B	H	$(CH_2)_2CONH(CH_2)_2Ph$	H	-	oil	$C_{38}H_{38}N_4O_2S_2$	HRFABMS
5	B	H	$(CH_2)_2CONHCH_2Ph\{4\text{-}COOMe\}$	H	-	151-153	$C_{40}H_{38}N_4O_6S_2$	C,H,N,S

TABLE 1 (cont'd)

	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
6	B	H	$(CH_2)_2CONHCH_2Ph\{4\text{-}COOH\}$	H	-	135.5-138.5 (dec)	$C_{38}H_{34}N_4O_6S_2 \cdot H_2O$	C,H,N,S
7	B	H	$(CH_2)_2CONHCH_2Ph\{3\text{-}OH, 4\text{-}COOMe\}$	H	-	183-185	$C_{40}H_{38}N_4O_8S_2$	C,H,N,S
8	B	H	$(CH_2)_2CONHCH_2Ph\{3\text{-}OH, 4\text{-}COOH\}$	H	-	160-163.5 (dec)	$C_{38}H_{34}N_4O_8S_2 \cdot H_2O$	C,H,N,S
9	B	H	$(CH_2)_2CONHPh$	H	-	114 (dec)	$C_{34}H_{30}N_4O_2S_2 \cdot 0.5H_2O$	C,H,N,S
0	B1	H	NHAc	H	-	140-144[#] (dec)	$C_{40}H_{40}N_6O_4S_2 \cdot 0.5H_2O$	C,H,N,S
					-	154.5-157.5[#] (dec)	$C_{40}H_{40}N_6O_4S_2$	C,H,N,S
1	B1	H	$NHCOCF_3$	H	-	160-164 (dec)	$C_{40}H_{34}F_6N_6O_4S_2 \cdot 0.5H_2O$	C,H,N,S
2	B1	H	NH_2	H	-	147-150 (dec)	$C_{36}H_{36}N_6O_2S_2 \cdot 0.5H_2O$	C,H,N,S
3	B1	H	OAc	H	-	120-124 (dec)	$C_{40}H_{34}N_4O_6S_2$	C,H,N,S
4	B1	H	OH	H	-	120-125	$C_{36}H_{34}N_4O_4S_2$	C,H,N,S
5	B	H	$(CH_2)_3COOH$	H	-	141-143.5	$C_{24}H_{24}N_2O_4S_2 \cdot 0.5H_2O$	C,H,N,S
6	B	H	$(CH_2)_3COOH$	Me	-	106.5-109.5	$C_{26}H_{28}N_2O_4S_2 \cdot 2AcOH$	C,H,N,S
7	B	H	$(CH_2)_3COOMe$	H	-	91-93	$C_{26}H_{28}N_2O_4S_2$	C,H,N,S
8	B	H	$(CH_2)_3COOMe$	Me	-	112-113	$C_{28}H_{32}N_2O_4S_2$	C,H,N,S
9	B	H	$(CH_2)_3CONHCH_2Ph$	H	-	98.5-101	$C_{38}H_{38}N_4O_2S_2$	C,H,N,S
0	B	H	CONHPh	Me	-	187-188	$C_{32}H_{26}N_4O_2S_2$	C,H,N,S
1	B	H	CONHPh	Et	-	200-202	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
2	B	4-Cl	CONHPh	Me	-	225-228	$C_{32}H_{24}Cl_2N_4O_2S_2$	C,H,N,Cl
3	B	5-Cl	CONHPh	Me	-	214-216	$C_{32}H_{24}Cl_2N_4O_2S_2$	C,H,N,S
4	B	7-Cl	CONHPh	Me	-	232-234	$C_{32}H_{24}Cl_2N_4O_2S_2$	C,H,N,Cl
5	B	4-Me	CONHPh	Me	-	237-239	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
6	B	5-Me	CONHPh	Me	-	231-234	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S

TABLE 1 (cont'd)

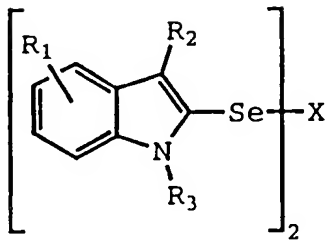
).	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
77	B	6-Me	CONHPh	Me	-	192-195	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
78	B	7-Me	CONHPh	Me	-	221-223	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
79	B	4-OMe	CONHPh	Me	-	225-228	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
80	B	5-OMe	CONHPh	Me	-	161-164	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
81	B	6-OMe	CONHPh	Me	-	197-200	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
82	B	7-OMe	CONHPh	Me	-	205-206	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
83	B	7-aza	CONHPh	Me	-	197-198	$C_{30}H_{24}N_6O_2S_2$	C,H,N,S
84	B	5-CF_3	CONHPh	Me	-	214-216	$C_{34}H_{24}F_6N_4O_2S_2$	C,H,N,S
85	B	6-Cl	CONHPh	Me	-	243-245	$C_{32}H_{24}Cl_2N_4O_2S_2$	C,H,N,S
86	B	5-NO_2	CONHPh	Me	-	236-240	$C_{32}H_{24}N_6O_6S_2 \cdot 2H_2O$	C,H,N
87	B	5-F	CONHPh	Me	-	205-207	$C_{32}H_{24}F_2N_4O_2S_2$	C,H,N,S
88	B	5-CN	CONHPh	Me	-	221-224	$C_{34}H_{24}N_6O_2S_2 \cdot 0.5H_2O$	C,H,N,S
89	B	5-Br	CONHPh	Me	-	219-221	$C_{32}H_{24}Br_2N_4O_2S_2$	C,H,N,S
90	B	4-OAc	CONHPh	Me	-	194	$C_{36}H_{30}N_4O_6S_2$	HRFABMS
91	B	5-OAc	CONHPh	Me	-	147-150	$C_{36}H_{30}N_4O_6S_2 \cdot 0.5H_2O$	C,H,N,S
92	B	5-OH	CONHPh	Me	-	185-187	$C_{32}H_{26}N_4O_4S_2 \cdot H_2O$	C,H,N
93	B	6-OAc	CONHPh	Me	-	219-222	$C_{36}H_{30}N_4O_6S_2$	C,H,N,S
94	B	6-OH	CONHPh	Me	-	185-187	$C_{32}H_{26}N_4O_4S_2$	HRMS
95	B	7-OAc	CONHPh	Me	-	212-214	$C_{36}H_{30}N_4O_6S_2 \cdot 0.5H_2O$	C,H,N,S
96	B	7-OH	CONHPh	Me	-	206-207	$C_{32}H_{26}N_4O_4S_2$	C,H,N,S
97	B	H	CONHMe	Me	-	162-165	$C_{22}H_{22}N_4O_2S_2$	HRMS[c]
98	B	H	$CONHCH_2Ph$	Me	-	145-147	$C_{34}H_{30}N_4O_2S_2$	C,H,N,S
99	B	H	SO_2Php-Me	H	-	230-233	$C_{30}H_{24}N_2O_4S_4$	C,H,N,S
00	B	H	COPh	Me	-	199-202	$C_{32}H_{24}N_2S_2O_2$	C,H,N,S

TABLE 1 (cont'd)

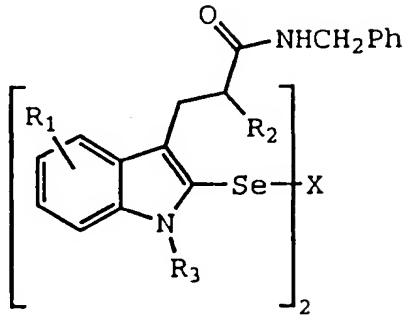
.	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
)1	B	H	COPhpCOOH	Me	-	241-246	$C_{34}H_{24}N_2S_2O_6 \cdot 1.5H_2O$	C,H
)2	B	H	COPhpCOOMe	Me	-	200-203	$C_{36}H_{28}N_2O_6S_2$	C,H,N,S
)3	B	H	Me	Me	-	113-115	$C_{20}H_{20}N_2S_2$	C,H,N,S
)4	B	H	CONHPh{4-COOMe}	Me	-	184-186	$C_{36}H_{30}N_4O_6S_2 \cdot H_2O$	C,H,N,S
)5	B	H	CONHPh{4-COOH}	Me	-	221	$C_{34}H_{26}N_4O_6S_2 \cdot 0.5H_2O$	C,H,N,S
)6	B	H	CONHPh{3-COOMe}	Me	-	193-195	$C_{36}H_{30}N_4O_6S_2$	C,H,N,S
)7	B	H	CONHPh{3-COOH}	Me	-	219-220	$C_{34}H_{26}N_4O_6S_2$	C,H,N,S
)8	B	H	CONHPh{2-COOMe}	Me	-	179-181	$C_{36}H_{30}N_4O_6S_2$	C,H,N,S
)9	B	H	CONHPh{2-COOH}	Me	-	184-186	$C_{34}H_{26}N_4O_6S_2$	C,H,N,S
10	B	H	$CONHCH_2Ph\{4-COOMe\}$	Me	-	178-180	$C_{38}H_{34}N_4O_6S_2$	C,H,N,S
11	B	H	$CONHCH_2Ph\{4-COOH\}$	Me	-	178-180	$C_{36}H_{30}N_4O_6S_2 \cdot 1.5H_2O$	C,H,N,S
12	B	H	$CONHCH_2COOH$	Me	-	196-198	$C_{24}H_{22}N_4O_6S_2$	C,H,N,S
13	B	H	CON(Me)Ph	Me	-	158-163	$C_{34}H_{31}N_4S_2O_2$	C,H,N,S
14	B	H	$CONHCH_2CH(OH)CH_2OH$	Me	-	198	$C_{26}H_{30}N_4O_6S_2$	C,H,N,S
15	B	H	$CONHCH_2CH_2NMe_2$	Me	-	163.5-165	$C_{28}H_{36}N_6O_2S_2$	C,H,N,S
16	B	H	CONH-4-pyridyl	Me	-	226-229	$C_{30}H_{24}N_6O_2S_2$	C,H,N,S
17	B	H	CONH-3-pyridyl	Me	-	257-260	$C_{30}H_{24}N_6O_2S_2$	C,H,N,S
18	B	H	$CONH_2$	Me	-	186-188	$C_{20}H_{18}N_4O_2S_2 \cdot 0.5H_2O$	C,H,N,S
19	B	H	$CONMe_2$	Me	-	96-102	$C_{24}H_{26}N_4O_2S_2 \cdot 0.5H_2O$	C,H,N
20	B	H	CN	Me	-	205-207	$C_{20}H_{14}N_4S_2$	C,H,N,S
21	B	H	COMe	Me	-	178.5-179.5	$C_{22}H_{20}N_2O_2S_2 \cdot 0.5H_2O$	C,H,N,S
22	B	H	CONH-2-pyridyl	Me	-	270-272	$C_{30}H_{24}N_6O_2S_2 \cdot 0.25H_2O$	C,H,N,S
23	B	H	CONH-furyl	Me	-	175-176	$C_{28}H_{20}N_2O_4S_2$	
24	B	H	CONH-thienyl	Me	-	183 (DEC)	$C_{28}H_{22}N_4O_4S_2 \cdot 0.5H_2O$	C,H,N

TABLE 1 (cont'd)

.	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
25	B	H	$CONHCH_2Ph$	H	-	203-205	$C_{32}H_{26}N_4O_2S_2$	C,H,N,S
26	B	H	CONHPh	H	-	220-222.5	$C_{30}H_{22}N_4O_2S_2$	C,H,N,S
27	B	H	CONHMe	H	-	232-236	$C_{20}H_{18}N_4O_2S_2$	C,H,N,S
28	B	H	CONHPh	$(CH_2)_3NMe_2$	-	165	$C_{28}H_{36}N_6O_2S_2$	C,H,N,S



D



D1

29	D	H	COOt-Bu	CH_3	-	187-189	$C_{28}H_{32}N_2O_4Se_2 \cdot 0.2H_2O$	C,H,N
30	D	H	COOH	CH_3	-	174 (dec)	$C_{20}H_{16}N_2O_4Se_2 \cdot 0.1H_2O$	C,H,N
31	D	H	CONHMe	CH_3	-	225-230 (dec)	$C_{22}H_{22}N_4O_2Se_2 \cdot 0.9H_2O$	C,H,N
32	D	H	$CONH(CH_2)_2NEt_2$	CH_3	-	160-164	$C_{32}H_{44}N_6O_2Se_2 \cdot 2.0HCl \cdot 1.7H_2O$	C,H,N,Cl^-

TABLE 1 (cont'd)

.	Formula	R_1	R_2	R_3	X	mp (°C)	Molecular Formula	Analysis[a]
33	D	H	$CONHCH_3$	H	-	272-275	$C_{20}H_{18}N_4O_2Se_2 \cdot 0.9H_2O$	C,H,N
34	D	H	$CONH(CH_2)_2NEt_2$	H	-	257-259 (dec)	$C_{30}H_{40}N_6O_2Se_2 \cdot 2.0HCl \cdot H_2O$	C,H,N
35	D	H	$CONHCH_3$	$(CH_2)_2NEt_2$	-	156-157	$C_{32}H_{44}N_6O_2Se_2 \cdot 0.5H_2O$	C,H,N
36	D1	H	NH_2[R-(R*,R*)]	H	-	172-174	$C_{36}H_{36}N_6O_2Se_2 \cdot 1.5H_2O$	C,H,N
37	D1	H	NH_2[S-(R*,R*)]	H	-	171 (dec)		

)iastereomers
ınalyses for all listed elements within ±0.4%
loncrystalline
ligh-resolution mass spectrum molecular ion

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EXAMPLES

The invention and the best mode for practicing the same are illustrated by the following Examples A-K.

EXAMPLE A

Preparation of Compounds 15, 17, 65, and 46 of Table 1 by the Method Outlined in Scheme 1

Concentrated HCl (16.6 mL) was added dropwise with stirring, over 10 minutes, to a solution of 4-(3-indolyl)butanoic acid [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOH$] (2.00 g) in DMSO (7.0 mL) at room temperature (method of Savige WE, Fontana A, J. Chem. Soc. Chem. Commun. 1976:599). After 15 minutes reaction, the mixture was diluted with water (80 mL) and extracted with EtOAc (4 x 100 mL). Removal of the solvent gave crude 4-(2-oxo-3-indolinyl)butanoic acid [III. $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOH$] (2.07 g, 96%) as a green-brown solid; mp (water) 169-171°C (Hinman RL, Bauman CP, J. Org. Chem. 1964;29:1206 record mp 170-171°C).

Acetyl chloride (10 mL) was added dropwise with stirring to an ice-cooled solution of the above crude acid [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOH$] (2.05 g) in dry MeOH (50 mL), and the mixture stirred at 20°C for 18 hours. The solvent was removed, and repeated evaporation from MeOH yielded a brown oil, which was dissolved in $CHCl_3$ (100 mL) and washed with water (2 x 100 mL). Removal of the solvent gave crude methyl 4-(2-oxo-3-indolinyl)butanoate [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (2.20 g) as an oil. A pure sample was obtained by chromatography on silica gel and elution with EtOAc/light petroleum (1:2) as a pale yellow oil.

^{1}H NMR ($CDCl_3$): δ 8.82 (1H, s, NH), 7.24 (1H, d, J = 7.7 Hz, ArH), 7.21 (1H, t, J = 7.8 Hz, ArH), 7.03 (1H, td, J = 7.6, 0.8 Hz, ArH), 6.91 (1H, d, J = 7.7 Hz, ArH), 3.65 (3H, s, $COOCH_3$), 3.49 (1H, t, J = 6.0 Hz, H-3), 2.34 (2H, t, J = 7.5 Hz, CH_2CO), 2.00, 1.72 (4H, 2xm, 3-CH_2CH_2).

^{13}C NMR ($CDCl^3$): δ 180.23 (s, CONH), 173.57 (s, $\underline{C}OOCH_3$), 141.54, 129.24 (2xs, Ar), 127.97, 124.11, 122.37, 109.80 (4xd, Ar), 51.53 (q, $COO\underline{C}H_3$), 45.74 (d, C3), 33.83, 29.79, 21.18 (3xt, $(\underline{C}H_3)_3CO$).

Analysis calculated for $C_{13}H_{15}NO_3 \cdot H_2O$ requires:
C, 6.45; H, 6.7; N, 5.6%.
Found: C, 64.4; H, 6.5; N, 5.7%.

A solution of the above crude ester [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (0.48 g) in dry dioxane (10 mL) was treated with P_2S_5 (0.26 g) and $NaHCO_3$ (0.36 g), then the mixture was stirred under nitrogen at 95°C for 1 hour. The resulting solution was concentrated under reduced pressure, and the residue was diluted with CH_2Cl_2 (100 mL) and filtered. The filtrate was washed with water, solvent was removed, and the residue (0.55 g) was chromatographed on silica gel (elution with CH_2Cl_2) to give crude methyl 4-(2-thioxo-3-indolinyl)butanoate [IV: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (17) (0.18 g, 35%); mp (benzene-light petroleum) 109-110°C.

^{1}N NMR ($CDCl_3$): δ 10.59 (1H, s, NH), 7.31 (1H, d, J = 7.4 Hz, ArH), 7.27 (1H, td, J = 7.7, 0.9 Hz, ArH), 7.14 (1H, td, J = 7.5, 0.9 Hz, ArH), 7.02 (1H, d, J = 7.7 Hz, ArH), 3.85 (1H, t, J = 5.5 Hz, H-3), 3.64 (3H, s, $COOCH_3$), 2.32 (2H, t, J = 7.5 Hz, CH_2CO), 2.26, 2.15, 1.67, 1.46 (4H, 4xm, 3-CH_2CH_2).

^{13}C NMR ($CDCl_3$): δ 207.80 (s, CSNH), 173.69 (s, $\underline{C}OOCH_3$), 143.27, 133.85 (2xs, ArH), 128.19, 124.17,

124.02, 110.12 (4xd, ArH), 57.36 (d, C-3), 51.61 (q, $COO\underline{C}H_3$), 33.92, 32.76, 20.41 (3xt, $(\underline{C}H_2)_3CO$).

Analysis calculated for $C_{13}N_{15}NO_2S$ requires:

C, 62.6; H, 6.1; N, 5.6; S, 12.9%.

Found: C, 62.8; H, 5.9; N, 5.7; S, 12.9%.

A solution of **17** (0.39 g) in MeOH was exposed to air for 13 days, then the solvent was removed. Chromatography of the residue on silica gel (elution with CH_2Cl_2) yielded bis[methylindolyl-3-butanoate-(2)]-disulfide [V: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (**67**) (0.31 g, 80%); mp (MeOH-dilute HCl) 91-93°C.

^{1}N NMR ($CDCl_3$): δ 8.19 (1H, s, NH), 7.57 (1H, d, J = 7.9 Hz, ArH), 7.28 (1H, d, J = 8.0 Hz, ArH), 7.24 (1H, ddd, J = 8.2, 7.1, 1.1 Hz, ArH), 7.12 (1H, ddd, J = 8.0, 6.9, 1.4 Hz, ArH), 3.56 (3H, s, $COOCH_3$), 2.67, 2.18 (2x2H, 2xt, J = 7.4 Hz, $C\underline{H}_2CH_2C\underline{H}_2CO$), 1.85 (2H, quin, J = 7.4 Hz, $CH_2C\underline{H}_2CH_2CO$).

^{13}C NMR ($CDCl_3$): δ 174.02 (s, $\underline{C}OOCH_3$), 137.29, 127.49, 125.99 (3xs, ArH), 124.21 (d, ArH), 123.70 (s, ArH), 119.95, 119.88, 111.08 (3xd, ArH), 51.42 (q, $COO\underline{C}H_3$), 33.45, 25.67, 23.95 (3xt, $(\underline{C}H_2)_3CO$).

Analysis calculated for $C_{26}H_{28}N_2O_4S_2$ requires:

C, 62.9; H, 5.7; N, 5.7; S, 12.9%.

Found: C, 62.6; H, 6.0; N, 5.5; S, 13.1%.

A mixture of **17** (0.26 g) in MeOH (10 mL) and K_2CO_3 (0.55 g) in water (3 mL) was stirred at room temperature for 2 days. $NaBH_4$ (100 mg) was then added, and the mixture stirred for 25 minutes, then diluted with water (100 mL) and extracted with CH_2Cl_2 (2 x 100 mL). The aqueous portion was acidified (to pH 3) with dilute HCl and extracted with EtOAc (3 x 100 mL). This extract was concentrated under reduced pressure, and the residue was crystallized from CH_2Cl_2-light petroleum to give 4-(2-thioxo-

3-indolinyl)butanoic acid [IV: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOH$] (**15**) (30 mg, 12%); mp 132-134°C.
^1H NMR (CD_3OD): δ 7.34 (1H, d, J = 7.4 Hz, ArH), 7.26 (1H, td, J = 7.7, 1.1 Hz, ArH), 7.12 (1H, td, J = 7.5, 0.8 Hz, ArH), 7.00 (1H, d, J = 7.8 Hz, ArH), 2.25 (2H, t, J = 7.5 Hz, $C\underline{H}_2COOH$), 2.24, 2.10, 1.55, 1.33 (4H, 4xm, 3-CH_2CH).
Analysis calculated for $C_{12}H_{13}NO_2S$ requires:
C, 61.3; H, 5.6; N, 6.0; S, 13.6%
Found: C, 61.1; H, 6.2; N, 6.1; S, 13.5%.

Similar hydrolysis of **67** (at 30°C for 6 hours, then 20°C for 1 day) gave bis[indolyl-3-butanoic acid-(2)]disulfide [V: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOH$] (**65**) (30 mg, 20%); mp (aqueous MeOH) 141-143.5°C.
^1H NMR (CD_3OD): δ 7.48 (1H, dt, J = 8.0, 0.8 Hz, ArH), 7.32 (1H, dt, J = 8.2, 0.7 Hz, ArH), 7.16 (1H, ddd, J = 8.1, 7.1, 1.1 Hz, ArH), 7.00 (1H, ddd, J = 8.0, 7.1, 0.8 Hz, ArH), 2.42 (2H, t, J = 7.6 Hz, CH_2CO), 1.93 (2H, t, J = 7.3 Hz, 3-CH_2), 1.58 (2H, quin, J = 7.5 Hz, $CH_2C\underline{H}_2CH_2CO$).
^{13}C NMR (CD_3OD): δ 177.52 (s, COOH), 139.31, 128.69, 126.69, 124.84 (4xs, ArH), 124.67, 120.48, 120.27, 112.34 (4xd, ArH), 34.39, 27.24, 24.82 (3xt, $(\underline{C}H_2)_3COOH$).
Analysis calculated for $C_{24}H_{24}N_2O_4S_2 \cdot H_2O$ requires:
C, 60.4; H, 5.2; N, 5.9; S, 13.4%.
Found: C, 60.4; H, 5.4; N, 5.9; S, 13.6%.

<u>Compounds 7, 9, 36 and 39 of Table 1</u>

Similar treatment of methyl 3-(3-indolinyl)-propanoic [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (0.93 g) with DMSO/HCl, followed by esterification with diazomethane and chromatography on silica gel, gave methyl 3-(2-oxo-3-indolyl)propanoate [III: R_1-R_3 = H,

R_2 = $(CH_2)_2COOMe$] (0.89 g, 89%) as a yellow oil (Julian PL, Printy HC, J. Am. Chem. Soc. 1953;75:5301-5305 report mp 79-80°C).

^1H NMR ($CDCl_3$): δ 8.75 (1H, s, NH), 7.22 (2H, m, ArH), 7.03 (1H, ddd, J = 7.8, 7.1, 1.1 Hz, ArH), 6.91 (1H, dd, J = 7.3, 1.3 Hz, ArH), 3.63 (3H, s, OCH_3), 3.54 (1H, t, J = 5.8 Hz, H-3), 2.61-2.20 (4H, m, 3-CH_2CH_2).

Analysis calculated for $C_{12}H_{13}NO_3$ requires:

M+ 219.0895.

HREIMS m/z Found: M+ 219.0898.

Treatment of this ester [III: R_1 = R_3 = H, R_2 = $(CH_2)_2COOMe$] (0.89 g) with P_2S_5 as above, followed by chromatography on silica gel, eluting with EtOAc/light petroleum (3:1), gave an oil (0.44 g). Crystallization from MeOH gave 2,2'-dithiobis[methyl 3-(3-indolyl)propanoate [V: R_1 = R_3 = H, R_2 = $(CH_2)_2COOMe$] (**39**) (61 mg, 6%); mp 162.5-164°C.

^1H NMR ($CDCl_3$): δ 8.21 (1H, s, NH), 7.55 (1H, dd, J = 8.0, 0.7 Hz, ArH), 7.25 (2H, m, ArH), 7.12 (1H, ddd, J = 8.0, 5.4, 2.6 Hz, ArH), 3.56 (3H, s, OCH_3), 2.98, 2.47 (2x2H, 2xt, J = 7.9 Hz, 3-CH_2CH_2).

^{13}C NMR ($CDCl_3$): δ 173.38 (s, $COOCH_3$), 137.25, 127.21, 125.80 (3xs, Ar), 124.30 (d, Ar), 122.79 (s, Ar), 120.10, 119.59, 111.21 (3xd, Ar), 51.56 (q, OCH_3), 34.97 (t, CH_2CO), 20.27 (t, 3-CH_2).

Analysis calculated for $C_{24}H_{24}N_2O_4S_2$ requires:

C, 61.5; H, 5.2; N, 6.0; S, 13.7%.

Found: C, 61.4; H, 5.3; N, 6.1; S, 13.7%.

Crystallization of the mother liquor residue from benzene/light petroleum gave methyl 3-(2-thioxo-3-indolinyl)propanoate [IV: R_1 = R_3 = H, R_2 = $(CH_2)_2COOMe$] (**9**) (0.24 g, 25%); mp (CH_2Cl_2/light petroleum) 96-98°C.

^{1}H NMR ($CDCl_3$): δ 9.83 (1H, s, NH), 7.29 (2H, m, ArH), 7.16 (1H, td, J = 7.5, 0.9 Hz, ArH), 6.99 (1H, d, J = 7.8 Hz, ArH), 3.91 (1H, t, J = 5.4 Hz, H-3), 3.60 (3H, s, OCH_3), 2.52 (2H, m, 3-CH_2), 2.42, 2.11 (2x1H, 2xm, CH_2CO).

^{13}C NMR ($CDCl_3$): δ 207.26 (s, CSNH), 173.37 (s, $COOCH_3$), 143.24, 133.08 (2xs, Ar), 128.43, 124.35, 124.09, 110.01 (4xd, Ar), 56.45 (d, C-3), 51.68 (q, OCH_3), 29.33, 28.19 (2xt, 3-CH_2CH_2).

Analysis calculated for $C_{12}H_{13}NO_2S$ requires:
C, 61.3; H, 5.6; N, 6.0; S, 13.6%.
Found: C, 61.4; H, 5.5; N, 6.0; S, 13.7%.

Hydrolysis of 9 with K_2CO_3/MeOH/H_2O as described above, followed by chromatography on silica gel, reduction with $NaBH_4$ and crystallization from CH_2Cl_2/isopropyl ether/light petroleum gave 3-(2-thioxo-3-indolinyl)propanoic acid [IV: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (7) (25 mg, 22%); mp 170-173°C.

^{1}H NMR (CD_3COCD_3): δ 11.48 (1H, s, NH), 7.43 (1H, d, J = 7.4 Hz, ArH), 7.30 (1H, t, J = 7.7 Hz, ArH), 7.15 (1H, t, J = 7.4 Hz, ArH), 7.11 (1H, d, J = 7.8 Hz, ArH), 3.90 (1H, t, J = 5.3 Hz, H-3), 2.49 (1H, m, CH_2CH_2CO), 2.37 (2H, m, CH_2CH_2CO), 2.11 (1H, m, CH_2CH_2CO).

^{13}C NMR (CD_3COCD_3): δ 208.48 (s, CSNH), 174.14 (s, COOH), 145.18, 134.55 (2xs, Ar), 129.05, 125.08, 124.30, 110.87 (4xd, Ar), 57.18 (d, C-3), 29,86, 29.25 (2xt, CH_2CH_2COOH).

Analysis calculated for $C_{11}H_{11}NO_2S$ requires:
C, 59.71; H, 5.01; N, 6.33%.
Found: C, 59.49; H, 4.97; N, 6.15%.

Aerial oxidation of 7 in MeOH at 20°C for 12 days, followed by dilution with water, gave

bis[indolyl-3-propanoic acid-(2)]disulfide [V: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (36) (30 mg, 30%); mp (aqueous MeOH) 118-120.5°C.

^1H NMR (CD_3OD): δ 7.47 (1H, dt, J = 8.0, 0.8 Hz, ArH), 7.30 (1H, dt, J = 8.1, 0.8 Hz, ArH), 7.15 (1H, ddd, J = 8.1, 7.1, 1.0 Hz, ArH), 7.00 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 2.74, 2.2 (2x2H, 2xt, J = 8.0 Hz, $(C\underline{H}_2)_2COOH$).

^{13}C NMR (CD_3OD): δ 176.95 (s, COOH), 139.26, 128.26 126.65 (3xs, Ar), 124.69 (d, Ar), 123.66 (s, Ar), 120.36, 120.20, 112.41 (3xd, Ar), 36.29, 21.22 (2xt, $(\underline{C}H_2)_2COOH$).

Analysis calculated for $C_{22}H_{20}N_2O_4S_2 \cdot H_2O$ requires:
C, 57.6; H, 4.8; N, 6.1; S, 14.0%.
Found: C, 57.6; H, 5.0; N, 6.1; S, 13.9%.

Compounds 3 and 27 of Table 1

Similar reaction of methyl 2-(2-oxo-3-indolinyl)-acetate [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$: Takase S, Uchida I, Tanaka H, Aoki H, Tetrahedron 1986;42:5879] (0.13 g) with P_2S_5 gave methyl 2-(2-thioxo-3-indolinyl)acetate [IV: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (3) (50 mg, 36%); mp (MeOH) 150-152°C.

^1N NMR ($CDCl_3$): δ 10.36 (1H, s, NH), 7.29 (1H, d, J = 7.6 Hz, ArH), 7.27 (1H, t, J = 7.8 Hz, ArH), 7.11 (1H, t, J = 7.6 Hz, ArH), 7.00 (1H, d, J = 7.8 Hz, ArH), 4.14 (1H, dd, J = 8.4, 4.2 Hz, H-3), 3.72 (3H, s, $COOCH_3$), 3.35 (1H, dd, J = 17.0, 4.2 Hz, CH_2CO), 2.88 (1H, dd, J = 17.0, 8.5 Hz, CH_2CO).

^{13}C NMR ($CDCl_3$): δ 206.59 (s, CSNH), 171.53 (s, $\underline{C}OOCH_3$), 143.10, 133.53 (2xs, ArH), 128.45, 124.20, 124.12, 110.07 (4xd, ArH), 53.53 (d, C3), 52.02 (q, $COO\underline{C}H_3$), 37.94 (t, CH_2).

Analysis calculated for $C_{11}H_{11}NO_2S$ requires:
C, 59.7; H, 5.0; N, 6.3; S, 14.5%.
Found: C, 59.9; H, 5.3; N, 6.4; S, 14.4%.

A solution of **3** (0.10 g) in benzene-light petroleum (1:1, 30 mL) exposed to air for 2 days gave a quantitative yield of bis[methylindolyl-3-acetate-(2)]-disulfide [V: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (Compound **27** of Table I); mp (benzene/light petroleum) 160-162°C.

^1N NMR ($CDCl_3$): δ 8.69 (1H, s, NH), 7.52 (1H, dd, J = 8.2, 0.6 Hz, ArH), 7.21 (1H, ddd, J = 8.2, 6.6, 1.1 Hz, ArH), 7.12 (2H, m, ArH), 3.83 (2H, s, CH_2CO), 3.71 (3H, s, $COOCH_3$).
^{13}C NMR ($CDCl_3$): δ 172.54 (s, $\underline{C}OOCH_3$), 137.20, 127.19, 127.03 (3xs, ArH), 124.26, 120.31, 119.45 (3xd, ArH), 116.23 (s, ArH), 111.41 (d, ArH), 52.25 (q, OCH_3), 30.51 (t, $\underline{C}H_2CO$).
Analysis calculated for $C_{22}H_{20}N_2O_4S_2$ requires:
C, 60.0; H, 4.6; N, 6.4; S, 14.6%.
Found: C, 60.0; H, 4.8; N, 6.3; S, 14.4%.

Additional amounts of **27** were also obtained from the mother liquors of the P_2S_5 reaction.

Compounds 8, 11, 37, and 40 of Table 1

A solution of 18-crown-6 (0.44 g), potassium t-butoxide (2.20 g) and methyl 3-(3-indolyl)propanoate [II: $R_1 = R_3 = H$; $R_2 = (CH_2)_2COOMe$] (3.24 g) in dry benzene (20 mL) was stirred at 20°C for 15 minutes, then cooled in ice. A solution of CH_3I (3.42 g) in benzene (10 mL) was added, then the flask was sealed and the mixture stirred at 20°C for 1 day (method of Guida WC, Mathre DJ, J. Org. Chem. 1980;45:3172). The resulting solution was filtered to remove salts, washing with CH_2Cl_2, then the combined filtrates washed

with water and the solvents removed. Chromatography on silica gel, eluting with CH_2Cl_2/light petroleum (1:1), gave methyl 3-(1-methyl-3-indolyl)propanoate [II: R_1 = H; R_3 = Me; R_2 = $(CH_2)_2COOMe$] (1.90 g, 52%) as a colorless oil (Snyder HR, Eliel EL, J. Am. Chem. Soc. 1949;71:663-669 report oil, $bp_{0.25}$ 180-190°C).

^1H NMR ($CDCl_3$): δ 7.58 (1H, dt, J = 7.7, 0.9 Hz, ArH), 7.28 (1H, dt, J = 7.9, 1.3 Hz, ArH), 7.21 (1H, ddd, J = 8.1, 6.7, 1.3 Hz, ArH), 7.10 (1H, ddd, J = 7.9, 6.5, 1.5 Hz, ArH), 6.86 (1H, s, H-2), 3.73, 3.67 (2x3H, 2xs, NCH_3, OCH_3), 3.09, 2.70 (2x2H, 2xt, J = 7.6 Hz, 3-CH_2CH_2).

Analysis calculated for $C_{13}H_{15}NO_2$ requires:
M+ 217.1103.

HREIMS *m/z* Found: M+ 217.1101.

Oxidation of the ester [II: R_1 = H; R_3 = Me; R_2 = $(CH_2)_2COOMe$] (1.85 g) with DMSO/HCl as above for 3 hours gave crude 3-(1-methyl-2-oxo-3-indolinyl)-propanoic acid [III: R_1 = H; R_2 = Me; R_3 = $(CH_2)_2COOH$] (2.08 g) as a colorless oil.

^1H NMR (CD_3OD): δ 7.31 (2H, m, ArH), 7.09 (1H, td, J = 8.0, 1.0 Hz, ArH), 6.98 (1H, d, J = 7.6 Hz, ArH), 3.56 (1H, t, J = 6.1 Hz, H-3), 3.20 (3H, s, NCH_3), 2.41-2.15 (4H, m, 3-CH_2CH_2).

^{13}C NMR (CD_3OD): δ 179.64 (s, COOH), 176.55 (s, $CONCH_3$), 145.52, 129.73 (2xs, Ar), 129.39, 125.00, 123.93, 109.64 (4xd, Ar), 45.79 (d, C-3), 31.01, 26.91 (2xt, 3-CH_2CH_2), 26.44 (q, NCH_3).

Analysis calculated for $C_{12}H_{13}NO_3$ requires:
M+ 219.0895.

HREIMS *m/z* Found: M+ 219.0897.

This was esterified with diazomethane as above, then the product chromatographed on silica gel. Elution with EtOAc/light petroleum (1:2) gave methyl

3-(1-methyl-2-oxo-3-indolinyl)propanoate [III: R_1 = H; R_2 = Me; R_3 = $(CH_2)_2COOMe$] (1.40 g, 70%) as a colorless oil.

^1H NMR ($CDCl_3$): δ 7.27 (2H, m, ArH), 7.06 (1H, td, J = 7.5, 0.8 Hz, ArH), 6.83 (1H, d, J = 7.7 Hz, ArH), 3.62 (3H, s, OCH_3), 3.50 (1H, t, J = 6.0 Hz, H-3), 3.20 (3H, s, NCH_3), 2.52-2.18 (4H, m, CH_2CH_2).

^{13}C NMR ($CDCl_3$): δ 177 23 (s, $CONCH_3$), 173.38 (s, $COOCH_3$), 144.36 (s, Ar), 128.20 (d, Ar), 128.11 (s, Ar), 123.92, 122.48, 108.06 (3xd, Ar), 51.64 (q, OCH_3), 44.36 (d, C-3), 30.12 (t, CH_2OCO), 26.14 (q, NCH_3), 25.64 (t, 3-CH_2).

Analysis calculated for $C_{13}H_{15}NO_3$ requires:

M+ 233.1052.

HREIMS *m/z* Found: M+ 233.1055.

Treatment of this ester [III: R_1 = H; R_2 = Me; R_3 = $(CH_2)_2COOMe$] (1.38 g) with P_2S_5 as above followed by chromatography on silica gel, eluting with CH_2CH_2/light petroleum (3:2), gave methyl 3-(1-methyl-2-thioxo-3-indolinyl)propanoate [IV: R_1 = H; R_3 = Me; R_2 = $(CH_2)_2COOMe$] (11) (1.40 g, 95%); mp (benzene/light petroleum) 71-73°C.

^1H NMR ($CDCl_3$): δ 7.35 (2H, m, ArH), 7.19 (1H, td, J = 7.5, 0.9 Hz, ArH), 7.00 (1H, d, J = 7.7 Hz, ArH), 3.92 (1H, t, J = 5.4 Hz, H-3), 3.63, 3.58 (2x3H, 2xs, NCH_3, OCH_3), 2.53 (2H, m, 3-CH_2), 2.34, 2.03 (2x1H, 2xm, CH_2CO).

^{13}C NMR ($CDCl_3$): δ 204.77 (s, $CSNCH_3$), 173.32 (s, $COOCH_3$), 145.89, 132.37 (2xs, Ar), 128.40, 124.31, 123.99, 109.51 (4xd, Ar), 56.26 (d, C-3), 51.61 (q, OCH_3), 31.35 (q, NCH_3), 29.31, 28.46 (2xt, 3-CH_2CH_2).

Analysis calculated for $C_{13}H_{15}NO_2S$ requires:

C, 62.6; H, 6.1; N, 5.6; S, 12.9%.

Found: C, 62.7; H, 6.3; N, 5.7; S, 13.0%.

Oxidation of (11) (0.70 g) with $FeCl_3$ (0.70 g) in $EtOAc/CH_2Cl_2$, chromatography of the product on silica gel, and elution with CH_2Cl_2 gave 2,2'-dithiobis[methyl 3-(1-methyl-3-indolyl)propanoate] [V: R_1 = H; R_2 = Me; R_2 = $(CH_2)_2COOMe$] (40) (0.38 g, 54%); mp (CH_2Cl_2/MeOH) 139-141.5°C.

^1H NMR ($CDCl_3$): δ 7.49 (1H, d, J = 8.0 Hz, ArH), 7.27 (1H, ddd, J = 8.3, 6.1, 0.9 Hz, ArH), 7.25 (1H, d, J = 8.1 Hz, ArH), 7.09 (1H, ddd, J = 8.0, 6.1, 1.9 Hz, ArH), 3.59, 3.53 (2x3H, 2xs, NCH_3, OCH_3), 2.76, 2.21 (2x2H, 2xt, J = 7.8 Hz, 3-CH_2CH_2).

^{13}C NMR ($CDCl_3$): δ 173.17 (s, $COOCH_3$), 138.49, 127.00, 126.09 (3xs, Ar), 124.14 (d, Ar), 123.77 (s, Ar), 119.68, 119.65, 109.87 (3xd, Ar), 51.39 (q, OCH_3), 35.09 (t, CH_2CO), 29.86 (q, NCH_3), 20.50 (t, 3-CH_2).

Analysis calculated for $C_{26}H_{28}N_2O_4S_2$ requires:
C, 62.9; H, 5.7; N, 5.7; S, 12.9%.
Found: C, 62.6; H, 5.6; N, 5.5; S, 13.0%.

A solution of (11) (0.53 g) in EtOH (10 mL) and 2N aqueous NaOH (3 mL) was stirred at 20°C for 80 minutes. The mixture was then diluted with water (100 mL) and extracted with CH_2Cl_2 (100 mL). The aqueous portion was adjusted to pH 2 with dilute HCl and extracted with EtOAc (3 x 120 mL). The EtOAc extracts were washed with water (150 mL) and the solvent removed under reduced pressure to give a yellow oil (0.48 g). This was redissolved in MeOH (7 mL) and 2 M aqueous NaOH (1 mL) and treated with NaBH (150 mg) for 5 minutes at 20°C. The mixture was then quenched with water and worked up as before to give a pale brown oil (0.46 g). Crystallization from CH/light petroleum gave 3-(1-methyl-2-thioxo-3-indolinyl)propanoic acid [R_1 = H; R_2 = Me; R_3 = $(CH_2)_2COOH$] (8) (0.32 g, 60%); mp 126-128.5°C.

^{1}H NMR ($CDCl_3$): δ 7.35 (2H, m, ArH), 7.18 (1H, td, J = 7.5, 0.9 Hz, ArH), 7.00 (1H, d, J = 7.8 Hz, ArH), 3.93 (1H, t, J = 5.3 Hz, H-3), 3.63 (3H, s, NCH_3), 2.51 (2H, m, 3-CH_2), 2.38 (1H, ddd, J = 16.1, 9.3, 6.7 Hz, CHCO), 2.06 (1H, ddd, J = 16.0, 9.8, 6.1 Hz, CHCO).

^{13}C NMR ($CDCl_3$): δ 204.61 (s, $CSNCH_3$), 178.41 (COOH), 145.88, 132.24 (2xs, Ar), 128.50, 124.38, 123.96, 109.57 (4xd, Ar), 56.05 (d, C-3), 31.37 (q, NCH_3), 29.16, 28.16 (2xt, 3-CH_2CH_2).

Analysis calculated for $C_{12}H_{13}NO_2S \cdot 0.25H_2O$ requires:
C, 60.1; H, 5.6; N, 5.8; S, 13.4%.
Found: C, 60.0; H, 5.6; N, 5.9; S, 13.4%.

Similar hydrolysis of **40** (0.37 g) in EtOH/2 M aqueous NaOH for 3 hours at 20°C gave, after workup, a yellow oil (0.30 g). Crystallization from AcOH gave 2,2'-dithiobis[3-(1-methyl-3-indolyl)propanoic acid] [V: R_1 = H; R_2 = $(CH_2)_2COOH$; R_3 = Me] (**37**) (73 mg, 20%); mp 158.5-160°C.

^{1}H NMR ($(CD_3)_2CO$): δ 7.59 (1H, d, J = 8.1 Hz, ArH), 7.39 (1H, d, J = 8.0 Hz, ArH), 7.27 (1H, ddd, J = 8.2, 7.1, 0.9 Hz, ArH), 7.07 (1H, ddd, J = 8.1, 7.1, 0.8 Hz, ArH), 3.60 (3H, s, NCH_3), 2.79, 2.31 (2x2H, 2xt, J = 7.9 Hz, 3-CH_2CH_2).

^{13}C NMR ($(CD_3)_2CO$): δ 173.75 (s, COOH), 139.61, 127.54, 127.06 (3xs, Ar), 125.08 (d, Ar), 125.02 (s, Ar), 120.55, 120.53, 110.03 (3xd, Ar), 35.56 (t, CH_2CO), 30.13 (q, NCH_3), 21.32 (t, 3-CH_2).

Analysis calculated for $C_{24}H_{24}N_2O_4S_2$ requires:
C, 61.5; H, 5.2; N, 6.0; S, 13.7%.
Found: C, 61.5; H, 5.2; N, 6.1; S, 13.6%.

Chromatography of the mother liquors on silica gel, then treatment with $NaBH_4$ as above and crystallization of the products from CH_2Cl_2/light

petroleum also gave 3-(1-methyl-2-thioxo-3-indolinyl)-propanoic acid (8) (0.12 g, 32%).

Compounds 16, 18, 66, and 68 of Table 1

N-Alkylation of methyl 4-(3-indolyl)butanoate [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (2.14 g), with 18-crown-6 (0.26 g), potassium t-butoxide/CH_3I as above gave methyl 4-(1-methyl-3-indolyl)butanoate [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$, $R_3 = Me$] (0.92 g, 40%) as a brown oil, which was used directly.

^1H NMR ($CDCl_3$): δ 7.58 (1H, dt, J = 7.9, 0.9 Hz, ArH), 7.28 (1H, d, J = 8.2 Hz, ArH), 7.21 (1H, ddd, J = 8.1, 7.0. 1.1 Hz, ArH), 7.09 (1H, ddd, J = 8.0, 7.0, 1.0 Hz, ArH), 6.84 (1H, s, ArH), 3.74 (3H, s, NCH_3), 3.66 (3H, s, $COOCH_3$), 2.79, 2.38 (2x2H, 2xt, J = 7.4 Hz, $C\underline{H}_2CH_2C\underline{H}_2CO$), 2.03 (2H, quin, J = 7.4 Hz, $CH_2C\underline{H}_2CH_2CO$).

^{13}C NMR ($CDCl_3$): δ 174.21 (s, $\underline{C}OOCH_3$), 137.08, 127.84 (2xs, ArH), 126.34, 121.50, 118.98, 118.62 (4xd, ArH), 114.07 (s, ArH), 109.13 (d, ArH), 51.44 (q, $COO\underline{C}H_3$), 33.68 (t, $\underline{C}H_2CO$), 32.55 (q, NCH_3), 25.58, 24.41 (2xt, 3-CH_2CH_2).

HREIMS *m/z* Found: M+ 231.1259.

4-(3-Indolyl)butanoic acid (1.04 g, 52%) was recovered by dissolving the filtered precipitates from the above reaction in water and acidifying; mp 124-126°C (Jackson RW, Manske RH, J. Am. Chem. Soc. 1930;52:5029 record mp 124°C).

Reaction of the ester [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$, $R_3 = Me$] with DMSO/HCl as above gave crude 4-(1-methyl-2-oxo-3-indolinyl)butanoic acid [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$, $R_3 = Me$] (0.84 g, 91% yield) as a brown oil.

^1H NMR ($CDCl_3$): δ 7.28 (1H, td, J = 7.7, 0.9 Hz, ArH), 7.25 (1H, d, J = 7.7 Hz, ArH), 7.06 (1H, td, J = 7.5,

0.9 Hz, ArH), 6.83 (1H, d, J = 7.8 Hz, ArH), 3.47 (1H, t, J = 5.9 Hz, H-3), 3.21 (3H, s, NCH_3), 2.37 (2H, t, J = 7.4 Hz, CH_2CO), 2.00, 1.69 (2x2H, 2xm, 3-CH_2CH_2).

An ice-cooled solution of the above crude oxoacid [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$, $R_3 = Me$] (0.84 g) in ether (10 mL) was treated, dropwise with stirring, with an ethereal solution of diazomethane (from N-nitrosomethylurea, 1.2 g). After 30 minutes at 20°C, the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (elution with EtOAc/light petroleum (1:2)) to give methyl 4-(1-methyl-2-oxo-3-indolinyl)butanoate [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$, $R_3 = Me$] (0.64 g, 72%); mp (EtOAc/light petroleum) 69-71°C.

^1H NMR ($CDCl_3$): δ 7.28 (1H, t, J = 7.8 Hz, ArH), 7.26 (1H, d, J = 7.6 Hz, ArH), 7.05 (1H, td, J = 7.6, 0.7 Hz, ArH), 6.82 (1H, d, J = 7.7 Hz, ArH), 3.64 (3H, s, $COOCH_3$), 3.44 (1H, t, J = 6.0 Hz, H-3), 3.20 (3H, s, NCH_3), 2.33 (2H, t, J = 7.5 Hz, CH_2CO), 1.98, 1.68 (2x2H, 2xm, 3-CH_2CH_2).

^{13}C NMR ($CDCl_3$): δ 177.52 (s, $\underline{C}ONCH_3$), 173.59 (s, $\underline{C}OOCH_3$), 144.38, 128.71 (2xs, ArH), 128.00, 123.84, 122.40, 108.02 (4xd, ArH), 51.54 (q, $COO\underline{C}H_3$), 45.26 (d, C-3), 33.89, 29.98 (2xt, $\underline{C}H_2CH_2\underline{C}H_2CO$), 26.15 (q, NCH_3), 21.30 (t, 3-$CH_2\underline{C}H_2$).

Analysis calculated for $C_{14}H_{17}NO_3$ requires:
C, 68.0; H, 6.9; N, 5.7%.
Found: C, 67.9; H, 6.7; N, 5.7%.

The above oxoester [III: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$, $R_3 = Me$] (0.90 g) was treated with P_2S_5 as above, followed by workup and chromatography on silica gel. Elution with CH_2Cl_2/light petroleum (3:2) gave methyl 4-(1-methyl-2-thioxo-3-indolyl)butanoate

[IV: R_1 = H, R_2 = $(CH_2)_3COOMe$, R_3 = Me] (18) (1.07 g, 79%); mp (benzene-light petroleum) 103-106°C.
^1H NMR ($CDCl_3$): δ 7.34 (2H, m, ArH), 7.19 (1H, td, J = 8.0, 0.9 Hz, ArH), 7.00 (dd, J = 8.0. 2.3).
Analysis calculated for $C_{14}H_{17}NO2S$ requires:
C, 63.9; H, 6.5; N, 5.3; S, 12.2%.
Found: C, 64.0; H, 6.4; N, 5.3; S, 12.3%.

A solution of 18 (0.47 g) in EtOAc (7 mL) was stirred with $FeCl_3$ (0.43 g) for 1 hour at 20°C, then worked up and chromatographed on silica gel. Elution with CH_2Cl_2 gave bis[methyl 1-methylindolyl-3-butanoate-(2)]disulfide [V: R_1 = H, R_2 = $(CH_2)_3COOMe$, R_3 = Me] (68) (0.40 g, 85%); mp (CH_2Cl_2/MeOH) 112-113°C.
^1H NMR ($CDCl_3$): δ 7.52 (1H, d, J = 8.0 Hz, ArH), 7.28 (1H, ddd, J = 8.2, 6.0. 1.0 Hz, ArH), 7.25 (1H, d, J = 8.0 Hz, ArH), 7.09 (1H, ddd, J = 8.0, 6.0. 1.9 Hz, ArH), 3.59, 3.55 (2x3H, 2xs, NCH_3, $COOCH_3$), 2.42, 2.07 (2x2H, 2xt, J = 7.4 Hz, $C\underline{H}_2CH_2C\underline{H}_2CO$), 1.68 (2H, quin, J = 7.4 Hz, $CH_2C\underline{H}_2CH_2CO$).
^{13}C NMR ($CDCl_3$): δ 173.82 (s, $\underline{C}OOCH_3$), 138.47, 127.23, 126.43, 124.74 (4xs, ArH), 124.05, 119.90, 119.49, 109.72 (4xd, ArH), 51.35 (q, $COO\underline{C}H_3$), 33.40 (t, $\underline{C}H_3CO$), 29.82 (q, NCH_3), 25.83, 24.17 (2xt, 3-CH_2CH_2).
Analysis calculated for $C_{28}H_{32}N_2O_4S_2$ requires:
C, 64.1; H, 6.1; N, 5.3; S, 12.2%.
Found: C, 63.9; H, 6.4; N, 5.3; S, 12.1%.

Hydrolysis of 18 with EtOH/H_2O/NaOH, followed by treatment with $NaBH_4$ and crystallization from CH_2Cl_2/light petroleum, as above, gave 4-(1-methyl-2-thioxo-3-indolyl)butanoic acid [IV: R_1 = H, R_2 = $(CH_2)_3COOH$, R_3 = Me] (16) (0.18 g, 44%); mp 144-146.5°C.

^{1}H NMR ($CDCl_3$): δ 7.34 (2H, m, ArH), 7.18 (1H, t, J = 7.6 Hz, ArH), 7.00 (1H, d, J = 7.7 Hz, ArH), 3.85 (1H, t, J = 5.5 Hz, H-3), 3.63 (3H, s, NCH_3), 2.34, 2.07 (2H, t, J = 7.6 Hz, CH_2CO), 2.28 2.18, 1.59, 1.40 (4x1H, 4xm, 3-CH_2CH_2).

^{13}C NMR ($CDCl_3$): δ 205.31 (s, $CSNCH_3$), 178.62 (s, COOH), 145.81, 133.06 (2xs, Ar), 128.20, 124.30, 123.86, 109.54 (4xd, Ar), 57.14 (d, C-3), 33.77, 33.01 (2xt, 3-$CH_2CH_2CH_2$), 31.42 (q, NCH_3), 20.11 (t, 3-CH_2CH_2).

Analysis calculated for $C_{13}H_{15}NO_2OS \cdot H_2O$ requires:
C, 61.6; H, 6.7; N, 5.5; S, 12.7%.
Found: C, 61.9; H, 6.3; N, 5.6; S, 12.8%.

Similar hydrolysis of **68** (0.40 g) gave, after workup, a yellow oil (0.37 g). Chromatography on silica gel, eluting with EtOAc/light petroleum (1:2) containing 1% AcOH, gave an oil (0.25 g). Crystallization from AcOH then gave 2,2'-dithiobis[4-(1-methyl-3-indolyl)butanoic acid] [V: R_1 = H, R_2 = $(CH_2)_3COOH$, R_3 = Me] (**66**) (0.17 g, 42%); mp 106.5-109.5°C.

^{1}H NMR ($CDCl_3$): δ 7.51 (1H, d, J = 8.0 Hz, ArH), 7.27 (2H, m, ArH), 7.08 (1H, ddd, J = 8.0, 6.0, 2.0 Hz, ArH), 3.55 (3H, s, NCH_3), 2.44 2.12 (2x2H, 2xt, J = 7.4 Hz, 3-$CH_2CH_2CH_2CO$), 1.68 (2H, quintet, J = 7.4 Hz, 3-$CH_2CH_2CH_2$).

^{13}C NMR ($CDCl_3$): δ 179.32 (s, COOH), 138.49, 127.49, 126.43, 124.56 (4xs, Ar), 124.14, 119,86, 119.62, 109.79 (4xd, Ar), 33.37 9t, CH_2CO), 29.86 (q, NCH_3) 25.59, 24.13 (2xt, 3-CH_2CH_2).

Analysis calculated for $C_{26}H_{28}N_2O_4S_2 \cdot 2CH_3COOH$ requires:
C, 58.4; H, 5.9; N, 4.5; S, 10.4%.
Found: C, 58.4; H, 5.9; N, 4.5; S, 10.6%.

EXAMPLE B

Preparation of Compounds 1, 29, 30, and 31 of Table 1 by the Method Outlined in Scheme 2

A solution of purified S_2Cl_2 (0.50 mL) in THF (20 mL) was added dropwise to a stirred, ice-cooled solution of 3-indolylacetic acid [II: $R_1 = R_3 = H$, $R_2 = CH_2COOH$] (2.20 g) in dry THF (30 mL) (method of Wieland T, Wieburg O, Fischer E, Korlein G, Annalen 1954;587:146). After 30 minutes at 20°C the solvent was removed, and the residue was crystallized from aqueous acetic acid to give a yellow solid (1.00 g). Recrystallization of this solid from aqueous MeOH, followed by further crystallization from EtOAc-benzene gave bis[indolyl-3-acetic acid-(2)]trisulfide [VI: $R_1 = R_3 = H$, $R_2 = CH_2COOH$, n = 3] (**30**) as a yellow powder (80 mg, 3%); mp 199-202°C.

^1H NMR (CD_3COCD_3): δ 10.18 (1H, s, NH), 7.59 (1H, m, ArH), 7.06 (2H, m, ArH), 6.82 (1H, m, ArH), 3.99 (2H, s, CH_2CO).

^{13}C NMR (CD_3COCD_3): δ 173.30 (s, COOH), 138.82, 128.26, 127.03 (3xs, ArH), 124.76, 120.60, 120.33 (3xd, ArH), 116.97 (s, ArH), 112.16 (d, ArH), 30.89 (t, $\underline{C}H_2CO$).

Analysis calculated for $C_{20}H_{16}N_2O_4S_3$ requires:
C, 54.1; H, 3.6; N, 6.3; S, 21.6%.
Found: C, 54.1; H, 3.8; N, 6.0; S, 21.2%.

The mother liquors from the above aqueous methanol crystallization were evaporated, and the resulting solid was recrystallized from CH_2Cl_2 to give bis[indolyl-3-acetic acid-(2)]disulfide [[VI: $R_1 = R_3 = H$, $R_2 = CH_2COOH$, n = 2] (**29**) as a yellow solid (0.19 g, 7%); mp 196-199°C (Wieland T, Wieburg O, Fischer E, Korlein G, Annalen 1954;587:146 record mp 208°C).

^{1}H NMR (CD_3COCD_3): δ 10.62 (1H, s, NH), 7.58 (1H, dd, J = 8.1, 0.6 Hz, ArH), 7.42 (1H, dt, J = 8.2. 0.8 Hz, ArH), 7.23 (1H, ddd, J = 8.2, 7.1, 0.9 Hz, ArH), 7.09 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 3.55 (2H, s, CH_2CO).

^{13}C NMR (CD_3COCD_3): δ 172.67 (s, COOH), 138.78, 128.33, 127.86 (3xs, ArH), 124.79, 120.72, 120.56 (3xd, ArH), 117.78 (s, ArH), 112.41 (d, ArH), 30.67 (t, $\underline{C}H_2CO$).

Analysis calculated for $C_{20}H_{16}N_2O_4S_2$ requires:
C, 58.2; H, 3.9; N, 6.8; S, 15.5%.
Found: C, 57.6; H, 4.4; N, 6.6; S, 15.3%.

Methylation of crude **30** with diazomethane as described above, followed by chromatography on silica gel, gave bis[methylindolyl-3-acetate-(2)]trisulfide [VI: $R_1 = R_3 = H$, $R_2 = CH_2COOMe$, n = 3] (**31**) (0.16 g, 47%); mp (CH_2Cl_2-light petroleum) 130-132°C.

^{1}H NMR ($CDCl_3$): δ 8.76 (1H, s, NH), 7.40 (1H, d, J = 8.0 Hz, ArH), 6.99 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 6.88 (1H, ddd, J = 8.2, 7.1, 0.9 Hz, ArH), 6.41 (1H, d, J = 8.2 Hz, ArH), 3.93 (2H, s, CH_2CO), 3.78 (3H, s, $COOCH_3$).

^{13}C NMR ($CDCl_3$): δ 172.93 (s, $\underline{C}OOCH_3$), 137.66, 127.02, 125.80 (3xs, ArH), 124.29, 120.06, 118.46 (3xd, ArH), 114.61 (s, ArH), 111.15 (d, ArH), 52.40 (q, $COO\underline{C}H_3$), 30.30 (t, $\underline{C}H_2CO$).

Analysis calculated for $C_{22}H_{20}N_2O_4S_3$ requires:
C, 55.9; H, 4.2; N, 5.9; S, 20.3%.
Found: C, 55.6; H, 4.4; N, 5.8; S, 19.9%.

Reduction of **29** with $NaBH_4/K_2CO_3$/MeOH as above gave 2-(2-thioxo-3-indolinyl)acetic acid [IV: $R_1 = R_3 = H$; $R_2 = CH_2COOH$] (**1**) (58 mg, 34%); mp (EtOAc/light petroleum) 166-168°C (Wieland T,

Wieburg O, Fischer E, Korlein G, Annalen 1954;587:146 record mp 170-171°C).

^{1}H NMR ($(CD_3)_2CO$): δ 11.51 (1H, s, NH), 7.39 (1H, d, J = 7.9 Hz, ArH), 7.29 (1H, td, J = 7.7, 0.8 Hz, ArH), 7.11 (2H, m, ArH), 4.02 (1H, dd, J = 3.9, 8.4 Hz, H-3), 3.36 (1H, dd, J = 17.2, 3.9 Hz, 3-CH), 2.83 (1H, dd, J = 17.2, 8.4 Hz, 3-CH).

Compounds 4 and 28 of Table 1

Methyl 2-(1-methyl-3-indolyl)acetate [II: R_1 = H; R_2 = CH_2COOMe; R_3 = Me] (Guida WC, Mathre DJ, J. Org. Chem. 1980;45:3172-3176) (1.18 g) was treated with S_2Cl_2 (0.25 mL) as above and the product then chromatographed on silica gel. Elution with CH_2Cl_2/light petroleum (2:1) and CH_2Cl_2 gave a yellow oil, from which crystallization with EtOAc/light petroleum gave 2,2'-monothiobis[methyl 2-(1-methyl-3-indolyl)acetate] [VI: R_1 = H, R_2 = CH_2COOMe; R_3 = Me; n = 1] (0.17 g, 13%); mp 155-156°C.

^{1}H NMR ($CDCl_3$): 7.54 (1H, d, *J* = 8.0 Hz, ArH), 7.22 (2H, m, ArH), 7.11 (1H, ddd, *J* = 8.0, 4.9, 3.0 Hz, ArH), 3.96 (2H, s, 3-CH_2), 3.61 (3H, s, OCH_3), 3.48 (3H, s, NCH_3).

^{13}C NMR ($CDCl_3$): 171.54 (s, *C*$OOCH_3$), 137.80, 126.80, 126.24 (3xs, Ar), 123.03, 119.92, 118.96 (3xd, Ar), 112.95 (s, Ar), 109.37 (d, Ar), 51.85 (q, OCH_3), 31.04 (t, 3-CH_2), 30.38 (q, NCH_3).

Analysis calculated for $C_{24}H_{24}N_2O_4S$ requires:
C, 66.1; H, 5.5; N, 6.4; S, 7.3%.
Found: C, 65.9; H, 5.6; N, 6.4; S, 7.4%.

Further crystallization of mother liquor fractions from benzene/light petroleum gave 2,2'-dithiobis[methyl 2-(1-methyl-3-indolyl)acetate] [VI: R_1 = H,

R_2 = CH_2COOMe; R_3 = Me; n = 2] (**28**) (0.16 g, 13%); mp 130-132.5°C.

^1H NMR ($CDCl_3$): 7.51 (1H, dt, *J* = 8.0, 0.8 Hz, ArH), 7.29 (2H, m, ArH), 7.12 (1H, ddd, *J* = 8.0, 6.0, 2.0 Hz, ArH), 3.57 (3H, s, OCH_3), 3.48 (3H, s, NCH_3), 3.33 (2H, s, 3-CH_2).

^{13}C NMR ($CDCl_3$): 171.44 (s, *C*$OOCH_3$), 138.42, 128.13, 126.38 (3xs, Ar), 124.37, 120.13, 120.08 (3xd, Ar), 117.48 (s, Ar), 109.94 (d, Ar), 51.79 (q, OCH_3), 30.57 (q, NCH_3), 29.96 (t, 3-CH_2).

Analysis calculated for $C_{24}H_{24}N_2O_4S_2$ requires:
C, 61.5; H, 5.1; N, 6.0; S, 13.7%.
Found: C, 61.4; H, 5.2; N, 6.0; S, 13.8%.

The remaining mother liquor was treated successively with $NaBH_4$ and $FeCl_3$ as above, to give an additional 0.36 g (26%) of **28**.

Reduction of **28** with $NaBH_4$ as above gave methyl 2-(1-methyl-2-thioxo-3-indolinyl)acetate [IV: R_1 = H; R_2 = CH_2COOMe; R_3 = Me] (**4**) (61%); mp (benzene/light petroleum) 68-70°C.

^1H NMR ($CDCl_3$): 7.34 (2H, m, ArH), 7.16 (1H, td, *J* = 7.5, 0.9 Hz, ArH), 7.01 (1H, d, *J* = 7.8 Hz, ArH), 4.15 (1H, dd, *J* = 8.7, 4.1 Hz, H-3), 3.71 (3H, s, OCH_3), 3.65 (3H, s, NCH_3), 3.40 (1H, dd, *J* = 17.0, 4.1 Hz, 3-CH), 2.83 (1H, dd, *J* = 17.0, 8.7 Hz, 3-CH).

^{13}C NMR ($CDCl_3$): 204.24 (s, *C*$SNCH_3$), 171.68 (s, *C*$OOCH_3$), 145.74, 132.95 (2xs, Ar), 128.47, 124.40, 123.96, 109.54 (4xd, Ar), 53.41 (d, C-3), 51.96 (q, OCH_3), 38.46 (t, 3-CH_2), 31.57 (q, NCH_3).

Analysis calculated for $C_{12}H_{13}NO_2S$ requires:
C, 61.3; H, 5.6; N, 6.0; S, 13.6%.
Found: C, 61.5; H, 5.8; N, 6.2; S, 13.9%.

Compounds 2 and 32 of Table 1

Similar treatment of 1-methyl-3-indolylacetic acid [II: R_1 = H, R_2 = CH_2COOH, R_3 = Me] (Guida WC, Mathre DJ, J. Org. Chem. 1980;45:3172; Kaestle KL, Anwer MK, Audhya TK, Goldstein G, Tetrahedron Lett. 1991;32:327) with S_2Cl_2 followed by chromatography on silica gel gave bis[1-methylindolyl-3-acetic acid-(2)]-disulfide [VI: R_1 = R_3 = H, R_2 = CH_2COOH, n = 2] (32) (0.10 g, 8%); mp (Me_2CO/light petroleum) 190-192.5°C (Wieland T, Wieburg O, Fischer E, Korlein G, Annalen 1954;587:146 record mp 190-191°C).

^1H NMR (CD_3COCD_3): δ 7.56 (1H, dt, J = 8.1, 0.9 Hz, ArH), 7.44 (1H, d, J = 8.3 Hz, ArH), 7.31 (1H, ddd, J = 8.2, 7.0, 1.2 Hz, ArH), 7.11 (1H, ddd, J = 8.0, 7.0, 0.9 Hz, ArH), 3.65 (3H, s, NCH_3), 3.23 (2H, s, CH_2CO).

^{13}C NMR (CD_3COCD_3): δ 172.21 (s, COOH), 139.52, 128.56, 127.45 (3xs, ArH), 125.21, 120.91, 120.74 (3xd, ArH), 119.38 (s, ArH), 111.04 (d, ArH), 30.81 (t, $\underline{C}H_2CO$), 30.31 (q, NCH_3).

Analysis calculated for $C_{22}H_{20}N_2O_2S_2$ requires:
C, 60.0; H, 4.6; N, 6.4; S, 14.5%.
Found: C, 59.4; H, 4.9; N, 6.4; S, 15.0%.

Reduction of 32 with $NaBH_4/K_2CO_3$/MeOH as above gave 2-(1-methyl-2-thioxo-3-indolinyl)acetic acid [IV: R_1 = H; R_2 = CH_2COOH; R_3 = Me] (2) (62 mg, 60%); mp (CH_2Cl_2/light petroleum) 150-153°C (Wieland T, Wieburg O, Fischer E, Korlein G, Annalen 1954;587:146 record mp 149-150°C).

^1H NMR ($CDCl_3$): δ 7.37 (2H, m, ArH), 7.18 (1H, t, J = 7.5 Hz, ArH), 7.02 (1H, d, J = 7.8 Hz, ArH), 4.14 (1H, dd, J = 8.6, 3.9 Hz, H-3), 3.65 (3H, s, NCH_3), 3.48 (1H, dd, J = 17.5, 4.0 Hz, 3-CH), 2.86 (1H, dd, J = 17.5, 8.7 Hz, 3-CH).

^{13}C NMR ($CDCl_3$): δ 203.88 (s, $CSNCH_3$), 176.31 (s, COOH), 145.67, 132.64 (2xs, Ar), 128.57, 124.52, 124.00, 109.59 (4xd, Ar), 53.07 (d, C-3), 38.33 (t, 3-CH_2), 31.59 (q, NCH_3).

Compounds 6 and 34 of Table 1

N-Benzyl 3-indolylacetamide [II: R_1 = R_3 = H, R_2 = $CH_2CONHCH_2Ph$] (Katritzky AR, J. Chem. Soc. 1955:2581) (1.48 g) was treated with S_2Cl_2 as above, and the product mixture was treated with $NaBH_4$ (ca. 0.7 g) in EtOH (10 mL) for 30 minutes at 20°C, then diluted with water (100 mL), acidified with dilute HCl and extracted in CH_2Cl_2 (2 x 100 mL) and EtOAc (100 mL). A sample from evaporation of the combined extracts was crystallized from EtOAc-light petroleum to give N-benzyl (2-thioxo-3-indolinyl)acetamide [IV: R_1 = R_3 = H, R_2 = $CH_2CONHCH_2Ph$] (6) (0.12 g, 7%); mp 193-195°C.

^1H NMR (CD_3SOCD_3): δ 12.64 (1H, s, NH), 8.50 (1H, t, J = 5.9 Hz, $NHCH_2$), 7.32 (2H, t, J = 7.3 Hz, ArH), 7.25 (3H, m, ArH), 7.11 (1H, d, J = 7.3 Hz, ArH), 7.00 (1H, t, J = 8.0 Hz, ArH), 6.53 (1H, m, ArH), 4.34, 4.28 (2x1H, 2xdd, J = 15.3, 5.9 Hz, $NHCH_2$), 4.04 (1H, dd, J = 9.5, 4.2 Hz, H-3), 3.10 (1H, dd, J = 15.3, 4.2 Hz, CH_2CO), 2.47 (1H, dd, J = 15.3, 9.5 Hz, CH_2CO).

^{13}C NMR (CD_3SOCD_3): δ 206.62 (s, CSNH), 169.41 (s, CONH), 143.97, 139.24, 134.36 (3xs, ArH), 128.22 (2xd, ArH), 127.95 (d, ArH), 127.36 (2xd, ArH), 126.77, 123.91, 123.09, 110.10 (4xd, ArH), 53.94 (d, C-3), 42.27, t, $NHCH_2$), 39.19 (t, CH_2CO).

Analysis calculated for $C_{17}H_{10}N_2OS$ requires:
C, 68.9; H, 5.4; N, 9.5; S, 10.8%.
Found: C, 68.8; H, 5.8; N, 9.5; S, 10.7%.

The remaining product mixture (1.60 g) was treated with $FeCl_3$ as above then chromatographed on silica gel to give a yellow oil (1.40 g). Crystallization from EtOAc/light petroleum then EtOAc gave 2,2'-dithiobis[N-benzyl 2-(3-indolyl)acetamide] [VI: $R_1 = R_3 = H$; $R_2 = CH_2CONHCH_2Ph$] (**34**) (0.36 g, 22%); mp 200.5-203.5°C.

^1H NMR $(CD_3)_2SO$): δ 11.57 (1H, s, CSNH), 8.45 (1H, t, J = 5.9 Hz, $NHCH_2$), 7.53 (1H, d, J = 8.0 Hz, ArH), 7.30 (1H, d, J = 8.2 Hz, ArH), 7.29-7.14 (6H, m, ArH), 7.01 (1H, t, J = 7.5 Hz, ArH), 4.19 (2H, d, J = 5.9 Hz, $NHCH_2$), 3.44 (2H, s, 3-CH_2).

^{13}C NMR $(CD_3)_2SO$): δ 170.08 (9s, CONH), 139.36, 137.42 (2xs, Ar), 128.12, 127.13 (4xd, Ar), 127.12, 126.82 (2xs, Ar), 126.63, 123.41, 119.67, 119.09 (4xd, Ar), 116.83 (s, Ar), 111.43 (d, Ar), 42.25 (t, $NHCH_2$), 31.73 (t, 3-CH_2).

Analysis calculated for $C_{34}H_{30}N_4O_2S_2$ requires:
C, 62.6; H, 6.1; N, 5.6; S, 12.9%.
Found: C, 62.7; H, 6.3; N, 5.7; S, 13.0%.

Compounds **13** and **47** of Table 1

Esterification of 3-(3-indolyl)propanoic acid [II: $R_1 = R_2 = H$, $R_3 = (CH_2)_2COOH$] (1.50 g) with diazomethane as above gave methyl 3-(3-indolyl)-propanoate [II: $R_1 = R_2 = H$, $R_3 = (CH_2)_2COOMe$] (1.62 g, 100%) as a light brown oil. This was stirred with benzylamine (5 mL) at 140°C for 4 hours (Katritzky AR, J. Chem. Soc. 1955:2581-2586) to give, after workup and chromatography on silica gel, N-benzyl 3-(3-indolyl)-propanamide [II: $R_1 = R_2 = H$; $R_3 = (CH_2)_2CONHCH_2Ph$] (1.81 g, 88%); mp (EtOAc/light petroleum) 125-126.5°C.

^1H NMR ($CDCl_3$): 8.05 (1H, s, NH), 7.59 (1H, d, J = 7.8 Hz, ArH), 7.34 (1H, d, J = 7.9 Hz, ArH), 7.24

(3H, m, ArH), 7.18 (1H, dd, J = 7.9, 7.2 Hz, ArH), 7.10 (1H, dd, J = 7.9, 7.2 Hz, ArH), 7.07 (2H, m, ArH), 6.93 (1H, d, J = 1.9 Hz, H-2), 5.64 (1H, t, J = 5.7 Hz, $NHCH_2$), 4.35 (2H, d, J = 5.7 Hz, 2 H, $NHCH_2$), 3.13, 2.59 (2x2H, 2xt, J = 7.3 Hz, 3-CH_2CH_2).

^{13}C NMR ($CDCl_3$): 172.54 (s, CONH), 138.20, 136.35 (2xs, Ar), 128.58, 127.66 (4xd, Ar), 127.35 (d, Ar), 127.08 (s, Ar), 122.04, 121.88, 119.35, 118.68 (4xd, Ar), 113.79 (s, Ar), 111.21 (d, Ar), 43.51 (t, $NHCH_2$), 37.42 (t, CH_2CO), 21.38 (t, 3-CH_2).

Analysis calculated for $C_{18}H_{18}N_2O$ requires:

C, 77.7; H, 6.6; N, 10.1%.

Found: C; 77.4; H, 6.5; N, 10.3%.

The above amide [II: R_1 = R_2 = H, R_3 = $(CH_2)_2CONHCH_2Ph$] (1.74 g) was treated with S_2Cl_2, and the product mixture was treated successively with $NaBH_4$ and $FeCl_3$ as above, then chromatographed on silica gel. Elution with EtOAc/light petroleum (2:1) gave 2,2'-monothiobis[N-benzyl 3-(3-indolyl)-propanamide] [VI: R_1 = R_2 = H; R_3 = $(CH_2)_2CONHCH_2Ph$; n = 1] (0.10 g, 6%); mp (CH_2Cl_2/light petroleum) 218-219°C.

^1H NMR $(CD_3)_2SO$): 11.01 (1H, s, CSNH), 8.38 (1H, t, J = 5.7 Hz, $NHCH_2$), 7.56 (1H, d, J = 7.9 Hz, ArH), 7.26-7.03 (7H, 2xm, ArH), 6.97 (1H, t, J = 7.5 Hz, ArH), 4.26 (2H, d, J = 5.5 Hz, $NHCH_2$), 3.22, 2.55 (2x2H, 2xt, J = 7.6 Hz, 3-CH_2CH_2).

Analysis calculated for $C_{36}H_{34}N_4O_2S \cdot H_2O$ requires:

C, 72.6; H, 5.9; N, 9.4; S, 5.4%.

Found: C, 72.7; H, 5.9; N, 9.6; S, 5.7%.

Further elution with EtOAc/light petroleum (1:1) gave a yellow oil (1.10 g) from which crystallization with benzene/CH_2Cl_2/light petroleum gave 2,2'-dithiobis[N-benzyl 3-(3-indolyl)propanamide]

[VI: $R_1 = R_2 = H$, $R_3 = (CH_2)_2CONHCH_2Ph$; n = 2] (**47**) (0.73 g, 38%); mp (CH_2Cl_2/light petroleum) 141-144°C.
^1H NMR ($CDCl_3$): 8.47 (1H, s, CSNH), 7.51 (1H, d, J = 7.9 Hz, ArH), 7.27-7.20 (4H, m, ArH), 7.13 (1H, ddd, J = 8.2, 7.1, 1.1 Hz, ArH), 7.00 (3H, m, ArH), 5.01 (1H, t, J = 5.7 Hz, $NHCH_2$), 4.16 (2H, d, t, J = 5.7 Hz, $NHCH_2$), 2.88, 1.87 (2x2H, 2xt, J = 7.7 Hz, 3-CH_2CH_2).
^{13}C NMR ($CDCl_3$): 171.93 (s, CONH), 138.30, 137.27 (2xs, Ar), 128.51, 127.78 (4xd, Ar), 127.30 (d, Ar), 127.07, 125.66 (2xs, Ar), 124.43 (d, Ar), 123.93 (s, Ar), 120.18, 119.94, 111.23 (3xd, Ar), 43.39 (t, $NHCH_2$), 37.09 (t, CH_2CO), 20.56 (t, 3-CH_2).
Analysis calculated for $C_{36}H_{34}N_4O_2S_2$ requires:
C, 69.9; H, 5.5; N, 9.1; S, 10.3%.
Found: C, 69.7; H, 5.6; N, 9.1; S, 10.5%.

Reduction of **47** with $NaBH_4$ as above gave a quantitative yield of N-benzyl 3-(2-thioxo-3-indolinyl)propanamide [IV: $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHCH_2Ph$] (**13**); mp (CH_2Cl_2) 149.5-151°C.
^1H NMR ($(CD_3)_2CO$): 11.46 (1H, s, CSNH), 7.45 (1H, t, J = 6.0 Hz, $NHCH_2$), 7.42 (1H, d, J = 7.9 Hz, ArH), 7.32-7.16 (6H, m, ArH), 7.13 (1H, td, J = 7.5, 0.9 Hz, ArH), 7.09 (1H, d, J = 7.8 Hz, ArH), 4.37, 4.33 (2x1H, 2xdd, J = 15.0, 6.0 Hz, $NHCH_2$), 3.87 (1H, t, J = 5.4 Hz, H-3), 2.56, 2.34, 2.04 (4H, 3xm, 3-CH_2CH_2).
^{13}C NMR ($(CD_3)_2CO$): 208.79 (s, CSNH), 172.23 (s, CONH), 145.20, 140.69, 134.88 (3xs, Ar), 129.14 (d, 2e, Ar), 128.93 (d, Ar), 128.33 (d, 2e, Ar), 127.62, 125.27, 124.22, 110.78 (4xd, Ar), 57.57 (d, C-3), 43.46 (t, $NHCH_2$), 31.87, 30.09 (2xt, 3-CH_2CH_2).
Analysis calculated for $C_{18}H_{18}N_2OS$ requires:
C, 67.7; H, 6.0; N, 8.8; S, 10.0%.
Found: C, 67.3; H, 5.9; N, 8.9; S, 10.5%.

Compound 69 of Table 1

3-(3-Indolyl)butanoic acid [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOH$] (1.10 g) was esterified with excess ethereal diazomethane to give methyl 4-(3-indolyl)butanoate [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_3COOMe$] (1.17 g, 100%); mp 73-75°C (Jackson RW, Manske RH, J. Am. Chem. Soc. 1930;52:5029 record mp 73-74°C). This was stirred with benzylamine (5 mL) at 150°C for 4 hours to give, after chromatography on silica gel (eluting with 1:4 EtOAc:CH_2Cl_2), *N*-benzyl-4-(3-indolyl)butanamide [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_3CONHCH_2Ph$] (1.43 g, 90%); mp (CH_2Cl_2/light petroleum) 123-124°C.

^1H NMR ($CDCl_3$): δ 8.05 (1H, br s, NH), 7.58 (1H, d, J = 7.9 Hz, ArH), 7.37-7.23 (6H, m, ArH), 7.18 (1H, ddd, J = 8.1, 7.1, 1.0 Hz, ArH), 7.10 (1H, ddd, J = 8.0, 7.0, 0.9 Hz, ArH), 6.95 (1H, d, J = 1.7 Hz, H-2), 5.68 (1H, br t, J = 5.7 Hz, $N\underline{H}CH_2$), 4.42 (1H, d, J = 5.7 Hz, $NHC\underline{H}_2$), 2.82 (2H, t, J = 7.3 Hz, 3-CH_2), 2.27 (2H, t, J = 7.5 Hz, CH_2CO), 2.09 (2H, pentet, J = 7.3 Hz, 3-$CHC\underline{H}_2$).

^{13}C NMR ($CDCl_3$): δ 172.79 (s, CONH), 138.35, 136.33 (2xs, Ar), 128.69, 127.84 (2d, 2x2C, Ar), 127.49 (d, Ar), 127.46 (s, Ar), 121.91, 121.50, 119.83, 118.3 (4xd, Ar), 115.57 (s, Ar), 111.10 (d, Ar), 43.58 (t, NCH_2), 36.15 (t, $\underline{C}H_2CO$), 26.06, 24.48 (2xt, 3-CH_2CH_2).

Analysis calculated for $C_{19}H_{20}N_2O$ requires:
C, 78.1; H, 6.9; N, 9.6%.
Found: C, 77.8; H, 6.8; N, 9.7%.

The above amide (1.38 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated with $NaBH_4$ as described above. The resulting oil was oxidized with 35% H_2O_2 (0.50 mL) in MeOH (10 mL) at 20°C for 20 minutes. Dilution with

water, extraction in CH_2Cl_2, and evaporation gave an oil which was chromatographed on silica gel. Elution with EtOAc/light petroleum (3:5) gave 2,2'-thiobis[*N*-benzyl-4-(3-indolyl)butanamide] [VI: n = 1; $R_1 = R_3 = H$, $R_2 = (CH_2)_3CONHCH_2Ph$] (0.14 g, 10%); mp (CH_2Cl_2/light petroleum) 105.5-108°C (dec).

^1H NMR ($CDCl_3$): δ 10.25 (1H, s, NH), 7.49 (1H, d, J = 8.0 Hz, ArH), 7.35-7.25 (6H, m, ArH), 7.11 (1H, ddd, J = 8.2, 7.0, 1.2 Hz, ArH), 7.01 (1H, ddd, J = 7.9, 7.0, 0.9 Hz, ArH), 5.75 (1H, t, J = 5.6 Hz, $N\underline{H}CH_2$), 4.38 (2H, d, J = 5.7 Hz, $NHC\underline{H}_2$), 3.07 (2H, t, J = 7.8 Hz, 3-CH_2), 2.38 (2H, t, J = 6.3 Hz, CH_2CO), 2.13 (2H, m, 3-$CH_2\underline{C}H_2$).

^{13}C NMR ($CDCl_3$): δ 173.49 (s, CONH), 138.12, 136.97 (2xs, Ar), 128.73, 127.93 (2xd, 2x2C, Ar), 127.56 (d, Ar), 127.48, 124.00 (2xs, Ar), 122.53 (d, Ar), 119.79 (s, Ar), 119.07, 118.60, 111.52 (3xd, Ar), 43.79 (t, NCH_2), 35.66 (t, CH_2CO), 25.77, 24.38 (2xt, 3-CH_2CH_2).

Analysis calculated for $C_{38}H_{38}N_4O_2S$ requires:
C, 74.3; H, 6.2; N, 9.1; S, 5.2%.
Found: C, 74.2; H, 6.1; N, 9.1; S, 5.0%.

Elution with EtOAc:light petroleum (1:1) gave 2,2'-dithiobis[*N*-benzyl-4-(3-indolyl)butanamide] (69) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_3CONHCH_2Ph$] (0.55 g, 36%); mp (CH_2Cl_2/benzene) 98.5-101°C (dec).

^1H NMR ($(CD_3)_2CO$): δ 10.48 (1H, s, NH), 7.58 (1H, d, J = 8.0 Hz, ArH), 7.38 (1H, d, J = 8.2 Hz, ArH), 7.37 (1H, m, $N\underline{H}CH_2$), 7.30-7.15 (6H, m, ArH), 7.03 (1H, ddd, J = 7.9, 7.3, 0.7 Hz, ArH), 4.30 (2H, d, J = 6.0 Hz, $NHC\underline{H}_2$), 2.67 (2H, t, J = 7.6 Hz, 3-CH_2), 2.09 (2H, t, J = 7.5 Hz, CH_2CO), 1.84 (2H, pentet, J = 7.5 Hz, 3-$CH_2\underline{C}H_2$).

^{13}C NMR ($(CD_3)_2CO$): δ 172.93 (s, CONH), 140.80, 138.83 (2xs, Ar), 129.12 (d, 2C, Ar), 128.46 (s, Ar), 128.35

(d, 2C, Ar), 127.58 (d, Ar), 126.71, 124.54, (2xs, Ar), 124.46, 120.60, 120.13, 112.36 (4xd, Ar), 43.43 (t, NCH_2), 36.34 (t, $\underline{C}H_2CO$), 27.75, 24.95 (2xt, 3-$CH_2\underline{C}H_2$).
Analysis calculated for $C_{38}H_{38}N_4O_2S_2$ requires:
C, 70.6; H, 5.9; N,8.7; S, 9.9%.
Found: C, 70.4; H, 6.0; N, 8.8; S, 9.8%.

Compound 35 of Table 1

3-Indolylacetonitrile [II: $R_1 = R_3 = H$, $R_2 = CH_2CN$] (1.00 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated with $NaBH_4$ as described above. Crystallization of the resulting oil from CH_2Cl_2 gave 2,2'-thiobis[3-indolylacetonitrile] [VI: n = 1; $R_1 = R_3 = H$, $R_2 = CH_2CN$] (0.11 g, 10%); mp 237-240°C (Piotrowska H, Serafin B, Wejroch-Matacz K, Rocz. Chem. 1975;49:635 record mp 242-244°C).
^1H NMR ($(CD_3)_2SO$): δ 11.61 (1H, s, NH), 7.65 (1H, d, J = 8.0 Hz, ArH), 7.37 (1H, d, J = 8.2 Hz, ArH), 7.20 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 7.10 (1H, ddd, J = 8.0, 7.1, 0.8 Hz, ArH), 4.26 (2H, s, 3-CH_2).
^{13}C NMR: δ 136.52, 125.99, 123.92 (3xs, Ar), 123.25, 119.78 (2xd, Ar), 118.67 (s, Ar), 118.48, 111.60 (2xd, Ar), 108.78 (s, 3-$CH_2\underline{C}N$), 12.98 (t, 3-CH_2).
Analysis calculated for $C_{20}H_{14}N_4S \cdot 0.5H_2O$ requires:
C, 68.4; H, 4.3; N, 16.0; S, 9.2%.
Found: C, 68.4; H,4.2; N, 16.2; S, 9.1%.

The mother liquor was oxidized with H_2O_2 in MeOH as above, then the resulting solid was chromatographed on silica gel, eluting with CH_2Cl_2, to give 2,2'-dithiobis[3-indolylacetonitrile] (35) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = CH_2CN$] (0.62 g, 52%); mp (CH_2Cl_2/MeOH) 168.5-169.5°C (Piotrowska H, Serafin B,

Wejroch-Matacz K, Rocz. Chem. 1975;49:635 record mp 169-170°C).

^{1}H NMR ($(CD_3)_2SO$): δ 11.90 (1H, s, NH), 7.67 (1H, d, J = 8.1 Hz, ArH), 7.42 (1H, d, J = 8.2 Hz, ArH), 7.28 (1H, ddd, J = 8.1, 7.1, 1.0 Hz, ArH), 7.14, (1H, ddd, J = 8.0, 7.1, 0.8 Hz, ArH), 3.69 (2H, s, 3-CH_2).

^{13}C NMR: δ 137.28, 126.36, 125.82 (3xs, Ar), 124.26, 120.03, 119.11, (3xd, Ar), 118.10 (s, Ar), 112.03 (d, Ar), 111.66 (s, 3-$CH_2\underline{C}N$), 12.56 (t, 3-CH_2).

Analysis calculated for $C_{20}H_{14}N_4S_2$ requires:
C, 64.2; H, 3.7; N, 15.0; S, 17.1%.
Found: C, 64.1; H, 3.9; N, 15.1; S, 17.0%.

Compound 48 of Table 1

3-Indolylpropionitrile [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2CN$] (Reppe W, Ufer H, German patent 698,273) (1.00 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$, then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with CH_2Cl_2, to give 2,2'-thiobis[3-indolylpropionitrile] [VI: n = 1; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CN$] (43 mg, 4%); mp (CH_2Cl_2/light petroleum) 204.5-207°C (Piotrowska H, Serafin B, Wejroch-Matacz K, Rocz. Chem. 1975;49:635 record mp 198-200°C).

^{1}H NMR ($(CD_3)_2SO$): δ 11.25 (1H, s, NH), 7.61 (1H, d, J = 7.9 Hz, ArH), 7.31 (1H, d, J = 7.8 Hz, ArH), 7.13 (1H, dd, J = 8.0, 7.1 Hz, ArH), 7.02 (1H, dd, J = 7.9, 7.1 Hz, ArH), 3.23, 2.71 (2x2H, 2xt, J = 7.2 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 136.65, 126.58, 124.04 (3xs, Ar), 122.65 (d, Ar), 120.36 (s, CN), 119.25, 118.79 (2xd, Ar), 116.32 (s, Ar), 111.31 (d, Ar), 20.60, 17.98 (2xt, 3-CH_2CH_2).

Further elution with CH_2Cl_2 gave 2,2'-dithiobis[3-indolylpropionitrile] (**48**) [VI: n = 2; R_1 = R_3 = H, R_2 = $(CH_2)_2CN$] (0.82 g, 69%); mp (CH_2Cl_2) 167-169°C (Piotrowska H, Serafin B, Wejroch-Matacz K, Rocz. Chem. 1975;49:635 record mp 165-167°C).

^1H NMR ($(CD_3)_2SO$): δ 11.71 (1H, s, NH), 7.59 (1H, d, *J* = 8.0 Hz, ArH), 7.38 (1H, dt, *J* = 8.2, 0.8 Hz, ArH), 7.22 (1H, ddd, *J* = 8.2, 7.1, 1.1 Hz, ArH), 7.04 (1H, ddd, *J* = 8.0, 7.1, 0.9 Hz, ArH), 2.57, 2.37 (2x2H, 2xt, *J* = 7.2 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 137.48, 126.16, 125.59 (3xs, Ar), 123.88 (d, Ar), 120.39, 119.87 (2xs, Ar,CN), 119.45, 111.64 (2xd, Ar), 19.80, 17.97 (2xt, 3-CH_2CH_2).

Compound 49 of Table 1

A solution of gramine (8.4 g) and methyl nitroacetate (11.5 g) in xylene (50 mL) was stirred under nitrogen at 90-100°C for 5 hours (method of Lyttle DA, Weisblat DI, J. Am. Chem. Soc. 1947;69:2118). Evaporation gave an oil which was chromatographed on silica gel, eluting with CH_2Cl_2:light petroleum (1:1), to give 3-(2-nitroethyl)indole [II: R_1 = R_3 = H, R_2 = $(CH_2)_2NO_2$] (4.44 g, 48%); mp (benzene/light petroleum) 57-59.5°C (Somei M, Karasawa Y, Kaneko C, Heterocycles 1981;16:941 record mp (MeOH) 54-55°C).

^1H NMR ($CDCl_3$): δ 8.05 (1H, br s, NH), 7.57 (1H, d, *J* = 7.9 Hz, ArH), 7.37 (1H, dt, *J* = 8.2, 0.8 Hz, ArH), 7.22 (1H, ddd, *J* = 8.1, 7.0, 1.1 Hz, ArH), 7.16 (1H, ddd, *J* = 7.9, 7.1, 0.9 Hz, ArH), 7.04 (1H, d, *J* = 2.4 Hz, H-2), 4.66 (2H, t, *J* = 7.3 Hz, 3-$CH_2\underline{CH_2}$), 3.49 (2H, td, *J* = 7.3, 0.6 Hz, 3-CH_2).

^{13}C NMR: δ 136.25, 126.67 (2xs, Ar), 122.56, 122.54, 119.91, 118.13, 111.45 (5xd, Ar), 110.05 (s, Ar), 75.73 (t, 3-$CH_2\underline{C}H_2$), 23.63 (t, 3-CH_2).

The above nitroethyl compound (1.50 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with CH_2Cl_2:light petroleum (4:3), to give 2,2'-thiobis[3-(2-nitroethyl)indole] [VI: n = 1; $R_1 = R_3 = H$, $R_2 = (CH_2)_2NO_2$] (49 mg, 3%); mp (CH_2Cl_2/light petroleum) 134.5-136°C.

^1H NMR ($(CD_3)_2SO$): δ 11.26 (1H, s, NH), 7.59 (1H, d, J = 7.9 Hz, ArH), 7.30 (1H, d, J = 8.1 Hz, ArH), 7.13 (1H, ddd, J = 8.1, 7.1, 0.9 Hz, ArH), 7.02 (1H, ddd, J = 7.9, 7.1, 0.8 Hz, ArH), 4.71 (2H, t, J = 7.3 Hz, 3-$CH_2\underline{C}H_2$), 3.57 (2H, t, J = 7.3 Hz, 3-CH_2).

^{13}C NMR: δ 136.59, 126.60, 124.20 (3xs, Ar), 122.56, 119.27, 118.43 (3xd, Ar), 113.37 (s, Ar), 111.24 (d, Ar), 75.11 (t, 3-$CH_2\underline{C}H_2$, 22.69 (t, 3-CH_2).

Analysis calculated for $C_{20}H_{18}N_4O_4S$ requires:
C, 58.5; H, 4.4; N, 13.7; S, 7.8%.
Found: C, 58.3; H, 4.7; N, 13.6; S, 8.0%.

Further elution as above gave 2,2'-dithiobis[3-(2-nitroethyl)indole] (49) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2NO_2$] (1.28 g, 73%); mp (CH_2Cl_2/light petroleum) 153-154°C.

^1H NMR ($(CD_3)_2SO$): δ 11.68 (1H, s, NH), 7.57 (1H, d, J = 8.0 Hz, ArH), 7.36 (1H, d, J = 8.2 Hz, ArH), 7.21 (1H, ddd, J = 8.1, 7.1, 0.9 Hz, ArH), 7.04 (1H, ddd, J = 7.9, 7.1, 0.8 Hz, ArH), 4.41 (2H, t, J = 7.2 Hz, 3-$CH_2\underline{C}H_2$), 2.97 (2H, t, J = 7.2 Hz, 3-CH_2).

^{13}C NMR: δ 137.37, 126.18, 125.95 (3xs, Ar), 123.76, 119.50, 119.08 (3xd, Ar), 117.39 (s, Ar), 111.59 (d, Ar), 75.05 (t, 3-$CH_2\underline{C}H_2$), 22.06 (t, 3-CH_2).

Analysis calculated for $C_{20}H_{18}N_4O_4S_2 \cdot 0.5H_2O$ requires:
C, 53.2; H, 4.2; N, 12.4; S, 14.2%.
Found: C, 53.4; H, 4.2; N, 12.6; S, 14.0%.

Compounds 14 and 50 of Table 1

DEPC (98%, 1.28 mL) was added to a stirred solution of 3-(3-indolyl)propanoic acid [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (1.30 g) and triethylamine (1.15 mL) in THF (15 mL) at 0°C. After 5 minutes the solution was saturated with ammonia gas, then the mixture was stirred at 20°C for 16 hours. The reaction was then quenched with water and extracted with EtOAc. Evaporation gave a solid, which was purified by chromatography on silica gel, eluting with EtOAc, to give 3-(3-indolyl)propanamide [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONH_2$] (1.09 g, 84%); mp (MeOH/water) 134-136°C (Crosby DG, Boyd JB, Johnson HE, J. Org. Chem. 1960;25:1826 record mp 131.5-133°C).

^1H NMR ($(CD_3)_2CO$): δ 9.95 (1H, s, NH), 7.58 (1H, dt, J = 8.2, 0.7 Hz, ArH), 7.36 (1H, dt, J = 8.1, 0.8 Hz, ArH), 7.13 (1H, m, H-2), 7.08 (1H, ddd, J = 8.1, 7.0, 1.1 Hz, ArH), 7.00 (1H, ddd, J = 8.0, 7.0, 1.0 Hz, ArH), 6.75, 6.12 (2xH, 2xbr s, $CONH_2$), 3.04 (2H, m, 3-CH_2), 2.05 (2H, m, 3-$CH_2\underline{C}H_2$).

^{13}C NMR: δ 174.87 (s, $CONH_2$), 137.75, 128.44 (2xs, Ar), 122.80, 122.02 (2xd, Ar), 119.30 (2xd, Ar), 115.67 (s, Ar), 112.08 (d, Ar), 37.05 (t, 3-$CH_2\underline{C}H_2$), 21.87 (t, 3-CH_2).

The above amide (1.03 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup

was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with EtOAc:light petroleum (3:1), to give firstly 2,2'-thiobis[3-(3-indolyl)-propanamide] [VI: n = 1; R_1 = R_3 = H, R_2 = $(CH_2)_2CONH_2$] (0.16 g, 14%); mp (EtOAc/light petroleum) 196.5-197.5°C.

^1H NMR ($(CD_3)_2SO$): δ 11.02 (1H, s, NH), 7.55 (1H, d, J = 8.0 Hz, ArH), 7.38 (1H, s, NH), 7.26 (1H, d, J = 8.1 Hz, ArH), 7.08 (1H, ddd, J = 8.0, 7.1, 0.8 Hz, ArH), 6.98 (1H, dd, J = 7.8, 7.1 Hz, ArH), 6.85 (1H, s, NH), 3.16, 2.46 (2x2H, 2xt, J = 7.7 Hz, 3-CH_2CH_2.

^{13}C NMR: δ 174.26 (s, $CONH_2$), 136.77, 126.82, 123.29 (3xs, Ar), 122.09, 118.82, 118.68 (3xd, Ar), 118.43 (s, Ar), 111.12 (d, Ar), 35.94 (t, 3-$CH_2\underline{C}H_2$), 20.58 (t, 3-CH_2).

Analysis calculated for $C_{22}H_{22}N_4O_2S$ requires:
C, 65.0; H, 5.4; N, 13.8; S, 7.9%.
Found: C, 64.8; H, 5.7; N, 13.6; S, 7.7%.

Further elution with EtOAc and EtOAc:EtOH (9:1) gave 2,2'-dithiobis[3-(3-indolyl)propanamide] (50) [VI: n = 2; R_1 = R_3 = H, R_2 = $(CH_2)_2CONH_2$] (0.90 g, 75%) as a yellow oil. A subsample crystallized from MeOH/dilute HCl as a solid which decomposed above 101°C.

^1H NMR $(CD_3)_2SO$): δ 11.37 (1H, s, NH), 7.55 (1H, d, J = 8.0 Hz, ArH), 7.32 (1H, d, J = 8.2 Hz, ArH), 7.16 (1H, t, J = 7.6 Hz, ArH), 7.00 (1H, t, J = 7.5 Hz, ArH), 6.94, 6.64 (2x1H, 2xs, $CONH_2$), 2.72, 2.14 (2x2H, 2xm, 3-CH_2CH_2).

^{13}C NMR: δ 173.48 (s, $CONH_2$), 137.42, 126.58, 125.09 (3xs, Ar), 123.29 (d, Ar), 122.65 (s, Ar), 119.53, 118.91, 111.46 (3xd, Ar), 36.48 (t, 3-$CH_2\underline{C}H_2$), 20.26 (t, 3-CH_2).

Analysis calculated for $C_{22}H_{22}N_4O_2S_2 \cdot 0.5H_2O$ requires:
C, 59.1; H, 5.2; N, 12.5; S, 14.3%.
Found: C, 59.1; H, 5.4; N, 12.2; S, 14.0%.

Reduction of (**50**) with $NaBH_4$ as above gave a quantitative yield of 3-(2-thioxo-3-indolinyl)-propanamide (**14**) [IV: $R_1 = R_2 = H$, $R_3 = (CH_2)_2CONH_2$]; mp (EtOAc) 160-163°C.

^1H NMR ($(CD_3)_2SO$): δ 12.63 (1H, s, NH), 7.38 (1H, d, J = 7.3 Hz, ArH), 7.27 (1H, t, J = 7.6 Hz, ArH), 7.22 (1H, s, NH), 7.12 (1H, t, J = 7.5 Hz, ArH), 7.00 (1H, d, J = 7.7 Hz, ArH), 6.70 (1H, s, NH), 3.84 (1H, t, J = 5.4 Hz, H-3), 2.38 (1H, m, 3-CH_2CH_2), 2.16-1.96 (2H, m, 3-CH_2CH_2), 1.77 (1H, ddd, J = 14.6, 10.3, 4.2 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 206.83 (s, CSNH), 173.37 ($CONH_2$), 144.11, 133.81 (2xs, Ar), 127.95, 124.11, 123.21, 110.03 (4xd, Ar), 56.35 (d, C-3), 30.12, 28.32 (2xt, 3-CH_2CH_2).

Analysis calculated for $C_{11}H_{12}N_2OS$ requires:
C, 60.0; H, 5.5; N, 12.7; S, 14.6%.
Found: C, 60.0; H, 5.5; N, 12.8; S, 14.3%.

Compound 51 of Table 1

DEPC (98%, 1.08 mL) was added to a stirred solution of 3-(3-indolyl)propanoic acid [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (1.10 g), triethylamine (1.94 mL) and methylamine hydrochloride (0.47 g) in THF (20 mL) at 0°C, then the mixture was stirred at 20°C for 20 hours. The reaction was then quenched with water and extracted with EtOAc. Evaporation gave an oil which was purified by chromatography on silica gel. Elution with EtOAc gave firstly foreruns, then *N*-methyl-3-(3-indolyl)-propanamide [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHMe$] (0.81 g, 69%); mp (CH_2Cl_2/light petroleum) 97.5-99°C

(Kononova VV, Vereshchagin AL, Polyachenka VM, Semenov AA, Khim.-Farm. Zh. 1978;12:30 record mp 97-99°C).

^{1}H NMR ($(CD_3)_2CO$): δ 9.97 (1H, s, NH), 7.56 (1H, dd, *J* = 8.0, 0.8 Hz, ArH), 7.36 (1H, dt, *J* = 8.1, 0.8 Hz, ArH), 7.11 (1H, m, H-2), 7.08 (1H, ddd, *J* = 8.1, 7.0, 1.1 Hz, ArH), 6.99 (1H, ddd, *J* = 7.8, 7.0, 1.0 Hz, ArH), 6.99 (1H, br s, $N\underline{H}CH_3$), 3.04 (2H, td, *J* = 7.7, 0.9 Hz, 3-CH_2), 2.68 (3H, d, *J* = 4.7 Hz, $NHC\underline{H}_3$), 2.51 (2H, t, *J* = 7.7 Hz, 3-$CH_2C\underline{H}_2$).

^{13}C NMR: δ 173.30 (s, CONH), 137.73, 128.42 (2xs, Ar), 122.80, 122.01, 119.31 (3xd, Ar), 115.62 (s, Ar), 112.08 (d, Ar), 37.67 (t, 3-$CH_2C\underline{H}_2$), 26.06 (q, NCH_3), 22.08 (t, 3-CH_2).

The above *N*-methylpropanamide (0.75 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with EtOAc, to give firstly 2,2'-thiobis[*N*-methyl-3-(3-indolyl)-propanamide] [VI: n = 1; R_1 = R_3 = H, R_2 = $(CH_2)_2CONHMe$] (0.13 g, 16%); mp (EtOAc/benzene/light petroleum) 120-123°C.

^{1}H NMR ($CDCl_3$): δ 10.50 (1H, s, NH), 7.54 (1H, d, *J* = 7.9 Hz, ArH), 7.31 (1H, d, *J* = 8.1 Hz, ArH), 7.14 (1H, ddd, *J* = 8.1, 7.1, 1.0 Hz, ArH), 7.04 (1H, ddd, *J* = 7.9, 7.0, 0.9 Hz, ArH), 5.31 (1H, br d, *J* = 4.9 Hz, $N\underline{H}CH_3$), 3.47 (2H, m, 3-CH_2), 2.80 (2H, m, 3-$CH_2C\underline{H}_2$), 2.60 (3H, d, *J* = 4.9 Hz, $NHC\underline{H}_3$).

^{13}C NMR: δ 174.25 (s, CONH), 137.17, 126.67, 125.39 (3xs, Ar), 122.51, 118.88, 118.58 (3xd, Ar), 117.62 (s, Ar), 111.43 (d, Ar), 36.01 (t, 3-$CH_2\underline{C}H_2$), 26.27 (q, NCH_3), 21.02 (t, 3-CH_2).

Analysis calculated for $C_{24}H_{26}N_4O_2S \cdot C_6H_6$ requires:
C, 70.3; H, 6.3; N, 10.9; S, 6.3%.
Found: C, 70.1; H, 6.2; N, 11.0; S, 6.0%.

Further elution with EtOAc gave 2,2'-dithiobis[*N*-methyl-3-(3-indolyl)propanamide] (**51**) [V: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHMe$] (0.29 g, 34%); mp (EtOAc/benzene/light petroleum) 162.5-164°C. ^1H NMR (CD_3CD): δ 7.50 (1H, dt, J = 8.1, 0.8 Hz, ArH), 7.33 (1H, dt, J = 8.2, 0.8 Hz, ArH), 7.18 (1H, ddd, J = 8.1, 7.0, 1.0 Hz, ArH), 7.02 (1H, ddd, J = 8.0, 7.1, 0.8 Hz, ArH), 2.71 (2H, m, 3-CH_2), 2.49 (3H, s, NCH_3), 2.02 (2H, m, 3-$CH_2\underline{CH_2}$). ^{13}C NMR: δ 175.76 (s, CONH), 139.27, 128.33, 127.01 (3xs, Ar), 124.80, (d, Ar), 123.92 (s, Ar), 120.48, 120.44, 112.48 (3xd, Ar), 38.44 (t, 3-$CH_2\underline{C}H_2$), 26.32 (q, NCH_3), 21.95 (t, 3-CH_2).
Analysis calculated for $C_{24}H_{26}N_4O_2S_2$ requires:
C, 61.8; H, 5.6; N, 12.0; S, 13.7%.
Found: C, 61.7; H, 5.7; N, 12.2; S, 13.7%.

<u>Compound **52** of Table 1</u>

A solution of 3-(3-indolyl)propanoic acid [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (0.70 g), triethylamine (5 mL) and methoxyamine hydrochloride (0.90 g) in THF (20 mL) was stirred at 20°C for 3 hours, then cooled to 0°C. DEPC (98%, 0.70 mL) was added, then the mixture was stirred at 20°C for 18 hours. The reaction was then quenched with water and extracted with EtOAc. Evaporation gave an oil which was purified by chromatography on silica gel. Elution with EtOAc:light petroleum (1:1) gave foreruns, then elution with EtOAc:light petroleum (3:1) gave *N*-methoxy-3-(3-indolyl)propanamide [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHOMe$] (0.50 g, 62%); mp (CH_2Cl_2/light

petroleum) 116-118°C (Kononova VV, Vereshchagin AL, Polyachenka VM, Semenov AA, Khim.-Farm. Zh. 1978;12:30 record mp 114-115°C).

^{1}H NMR ($(CD_3)_2SO$): δ 10.97, 10.77 (2x1H, 2xs, 2xNH), 7.51 (1H, d, *J* = 7.8 Hz, ArH), 7.32 (1H, d, *J* = 8.1 Hz, ArH), 7.09 (1H, s, H-2), 7.06 (1H, td, *J* = 8.0, 1.0 Hz, ArH), 6.97 (1H, td, *J* = 8.0, 0.9 Hz, ArH), 3.55 (3H, s, $NHOC\underline{H}_3$), 2.91, 2.30 (2x2H, 2xt, *J* =7.6 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 168.72 (s, CONH), 136.13, 126.87 (2xs, Ar), 122.14, 120.83, 118.21, 118.09 (4xd, Ar), 113.30 (s, Ar), 111.23 (d, Ar), 63.00 (q, OCH_3), 33.20 (t, 3-$CH_2\underline{C}H_2$), 20.53 (t, 3-CH_2).

The above N-methoxypropanamide (1.00 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with EtOAc:light petroleum (3:2), to give firstly 2,2'-thiobis[*N*-methoxy-3-(3-indolyl)propanamide] [VI: n = 1; R_1 = R_3 = H, R_2 = $(CH_2)_2CONHOMe$] (0.12 g, 11%); mp (EtOAc/light petroleum) 157.5-158.5°C.

^{1}H NMR ($(CD_3)_2SO$): δ 11.02, 10.95 (2x1H, 2xs, 2xNH), 7.53 (1H, d, *J* = 7.9 Hz, ArH), 7.25 (1H, d, *J* = 8.1 Hz, ArH), 7.09 (1H, t, *J* = 7.5 Hz, ArH), 6.99 (1H, t, *J* = 7.4 Hz, ArH), 3.52 (3H, s, $NHOC\underline{H}_3$), 3.17, 2.31 (2x2H, 2xt, *J* = 7.5 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 168.73 (s, CONH), 136.75, 126.79, 123.29 (3xs, Ar), 122.23 (d, Ar), 118.78 (d, 2C, Ar), 118.00 (s, Ar), 111.08 (d, Ar), 63.04 (q, OCH_3), 33.43 (t, 3-$CH_2\underline{C}H_2$), 20.46 (t, 3-CH_2).

Analysis calculated for $C_{24}H_{26}N_4O_4S$ requires:
C, 61.8; H, 5.6; N, 12.0; S, 6.9%.
Found: C, 61.6; H, 5.8; N, 12.2; S, 6.9%.

Elution with EtOAc gave 2,2'-dithiobis[*N*-methoxy-3-(3-indolyl)propanamide] (**52**) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHOMe$] (0.35 g, 31%); mp (EtOAc/light petroleum) 176-178°C.

^1H NMR ($(CD_3)_2SO$): δ 11.39, 10.73 (2x1H, 2xs, 2xNH), 7.51 (1H, d, J = 8.0 Hz, ArH), 7.32 (1H, d, J = 8.2 Hz, ArH), 7.16 (1H, t, J = 7.7 Hz, ArH), 7.00 (1H, t, J = 7.5 Hz, ArH), 3.41 (3H, s, $NHOC\underline{H}_3$), 2.65, 2.01 (2x2H, 2xt, J = 7.4 Hz, 3-$CH_2\underline{C}H_2$).

^{13}C NMR: δ 168.21 (s, CONH), 137.42, 126.52, 125.16 (3xs, Ar), 123.37 (d, Ar), 122.20 (s, Ar), 119.48, 118.96, 111.48 (3xd, Ar), 62.91 (q, OCH_3), 33.79 (t, 3-$CH_2\underline{C}H_2$), 20.09 (t, 3-CH_2).

Analysis calculated for $C_{24}H_{26}N_4O_4S_2$ requires:
C, 57.8; H, 5.2; N, 11.2; S, 12.9%.
Found: C, 57.6; H, 5.4; N, 11.3; S, 12.7%.

Compound **53** of Table 1

DEPC (98%, 1.28 mL) was added to a stirred solution of 3-(3-indolyl)propanoic acid [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (1.04 g) and triethylamine (1.15 mL) in THF (15 mL) at 0°C. After 5 minutes the solution was saturated with dimethylamine gas, then the mixture was stirred at 20°C for 16 hours. Workup as above and chromatography on silica gel, eluting with EtOAc, gave *N*,*N*-dimethyl 3-(3-indolyl)propanamide [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONMe_2$] (0.90 g, 76%); mp (CH_2Cl_2/light petroleum) 141-142°C (Avramenko VG, Suvorov NN, Mashkovskii MD, Mushulov PI, Eryshev BYa, Fedorova VS, Orlova IA, Trubitsyna TK, Khim.-Farm. Zh. 1970;4:10 record mp 139-140.5°C).

^1H NMR (CD_3OD): δ 7.53 (1H, dt, J = 7.9, 0.9 Hz, ArH), 7.32 (1H, dt, J = 8.1, 0.8 Hz, ArH) 7.07 (1H, ddd,

J = 8.1, 7.0, 1.1 Hz, ArH), 7.04 (s, H-2), 6.99 (1H, ddd, J = 7.9, 7.0, 0.9 Hz, ArH), 3.05 (2H, m, 3-CH_2), 2.88, 2.86 (2x3H, 2xs, $N(CH_3)_2$, 2.73 (2H, m, 3-$CH_2\underline{C}H_2$).
^{13}C NMR: δ 175.75 (s, $\underline{C}ON(CH_3)_2$), 138.20, 128.59 (2xs, Ar), 123.11, 122.36, 119.61, 119.24 (4xd, Ar), 115.16 (s, Ar), 112.26 (d, Ar), 37.89, 35.82 (2xq, $N(CH_3)_2$), 35.30 (t, 3-$CH_2\underline{C}H_2$), 22.32 (t, 3-CH_2).

The above dimethylpropanamide (0.82 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with EtOAc:light petroleum (3:2), to give firstly 2,2'-thiobis-[*N*,*N*-dimethyl-3-(3-indolyl)propanamide] [VI: n = 1; R_1 = R_3 = H, R_2 = $(CH_2)_2CONHMe_2$] (0.12 g, 14%); mp (EtOAc/light petroleum) 189-190°C.
^1H NMR ($CDCl_3$): δ 10.72 (br s, 1 H, NH), 7.55 (1H, d, J = 7.9 Hz, ArH), 7.24 (1H, d, J = 8.1 Hz, ArH), 7.10 (ddd, J = 8.0, 7.1, 0.9 Hz, 1 H, ArH), 7.02 (dd, J = 7.9, 7.1 Hz, 1 H, ArH), 3.47, 2.97 (2x2H, 2xm, 3-CH_2CH_2), 2.95, 2.91 (2x3H, 2xs, $N(CH_3)_2$).
^{13}C NMR: δ 173.36 (s, $\underline{C}ON(CH_3)_2$), 137.15, 126.92, 125.55 (3xs, Ar), 122.26, 118.68, 118.58 (3xd, Ar), 118.02 (s, Ar), 111.35 (d, Ar), 37.49, 35.74 (2xq, $N(CH_3)_2$), 32.14 (t, 3-$CH_2\underline{C}H_2$), 20.54 (t,3-CH_2).
Analysis calculated for $C_{26}H_{30}N_4O_2S$ requires:
C, 67.5; H, 6.5; N, 12.1; S, 6.9%.
Found: C, 67.4; H, 6.6; N, 12.0; S, 7.2%.

Elution with EtOAc gave 2,2'-dithiobis-[*N*,*N*-dimethyl-3-(3-indolyl)propanamide] (**53**) [VI: n = 2; R_1 = R_3 = H, R_2 = $(CH_2)_2CONMe_2$] (0.49 g, 52%); mp (EtOAc) 179-180°C.
^1H NMR (CD_3OD): δ 7.45 (1H, dt, J = 8.0, 0.8 Hz, ArH), 7.32 (1H, dt, J = 8.2, 0.8 Hz, ArH), 7.17 (1H, ddd,

J = 8.2, 7.1, 1.1 Hz, ArH), 7.01 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 2.72 (2H, m, 3-$C\underline{H}_2CH_2$), 2.71, 2.44 (2x3H, 2xs, $N(C\underline{H}_3)_2$), 2.09 (2H, m, 3-$CH_2C\underline{H}_2$).

^{13}C NMR: δ 174.68 (s, $\underline{C}ON(CH_3)_2$), 139.43, 128.26, 126.61 (3xs, Ar), 124.85 (d, Ar), 123.84 (s, Ar), 120.55, 120.28, 112.51 (3xd, Ar), 37.57 (q, NCH_3), 35.69 (t, 3-CH_2CH_2), 35.60 (q, NCH_3), 21.49 (t, 3-CH_2).

Analysis calculated for $C_{26}H_{30}N_4O_2S_2$ requires:
C, 63.2; H, 6.1; N, 11.3; S, 13.0%.
Found: C, 63.2; H, 6.2; N, 11.3; S, 13.1%.

Compound 54 of Table 1

DEPC (98%, 0.69 mL) was added to a stirred solution of 3-(3-indolyl)propanoic acid [II: R_1 = R_3 = H, R_2 = $(CH_2)_2COOH$] (0.70 g) and phenethylamine (1.1 mL) in THF (15 mL) at 0°C, then the mixture was stirred at 20°C for 3 hours. Workup and chromatography on silica gel, eluting with EtOAc/light petroleum (1:1) gave *N*-(2-phenylethyl)-3-(3-indolyl)-propanamide [II: R_1 = R_3 = H, R_2 = $(CH_2)_2CONH(CH_2)_2Ph$] (0.58 g, 54%); mp (EtOAc/light petroleum) 88-89°C.

^1H NMR ($CDCl_3$): δ 8.02 (1H, br s, NH), 7.58 (1H, d, J = 7.9 Hz, ArH), 7.36 (1H, d, J = 8.1 Hz, ArH), 7.24-7.15 (4H, m, ArH), 7.12 (1H, ddd, J = 7.9, 7.0, 0.8 Hz, ArH), 6.99 (2H, dd, J = 7.4, 1.7 Hz, ArH), 6.95 (1H, d, J = 2.2 Hz, H-2), 5.34 (1H, br t, J = 6.0 Hz, $N\underline{H}CH_2$), 3.44 (2H, q, J = 6.6 Hz, $NHC\underline{H}_2$), 3.09 (2H, t, J = 7.3 Hz, 3-CH_2), 2.66 (2H, t, J = 6.9 Hz, $NHCH_2C\underline{H}_2$), 2.52 (2H, t, J = 7.3 Hz, 3-$CHC\underline{H}_2$).

^{13}C NMR: δ 172.64 (s, CONH), 138.90, 136.38 (2xs, Ar), 128.71, 128.58 (2xd, 2x2C, Ar), 127.13 (s, Ar), 126.41, 122.10, 121.77, 119.37, 118.72 (5xd, Ar), 114.95 (s, Ar), 111.23 (d, Ar), 40.48, 37.42, 35.62 (3xt, 3-$CH_2\underline{C}H_2CONH(\underline{C}H_2)_2$), 21.35 (t, 3-$CH_2$).

Analysis calculated for $C_{19}H_{20}N_2O$ requires:
C, 78.1; H, 6.9; N, 9.6%.
Found: C, 77.9; H, 7.0; N, 9.6%.

The above phenylethylpropanamide (0.53 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with EtOAc:light petroleum (1:2), to give firstly 2,2'-thiobis[*N*-(2-phenylethyl)-3-(3-indolyl)-propanamide] [VI: n = 1; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONH(CH_2)_2Ph$] (0.13 g, 23%); mp (EtOAc/light petroleum) 120-121.5°C.

^1H NMR ($CDCl_3$): δ 10.69 (1H, s, NH), 7.55 (1H, d, J = 7.9 Hz, ArH), 7.35 (1H, d, J = 8.2 Hz, ArH), 7.17 (1H, ddd, J = 8.1, 7.1, 1.0 Hz, ArH), 7.08 (1H, ddd, J = 8.0, 0.9 Hz, ArH), 7.02 (1H, t, J = 7.4 Hz, ArH), 6.93 (2H, t, J = 7.4 Hz, ArH), 6.33 (2H, d, J = 7.2 Hz, ArH), 5.26 (1H, t, J = 5.9 Hz, $N\underline{H}CH_2$), 3.51 (2H, m, 3-CH_2), 3.14 (2H, q, J = 6.6 Hz, $NHC\underline{H}_2$), 2.77 (2H, m, 3-$CH_2C\underline{H}_2$), 1.92 (2H, t, J = 6.8 Hz, $NHCH_2C\underline{H}_2$).

^{13}C NMR: δ 173.62 (s, CONH), 138.20, 137.33 (2xs, Ar), 128.40, 128.36 (2xd, 2x2C, Ar), 126.76 (s, Ar), 126.16 (d, Ar), 125.51 (s, Ar), 122.78, 119.17, 118.70 (3xd, Ar), 117.57 (s, Ar), 111.70 (d, Ar), 40.49, 36.43, 35.46 (3xt, 3-$CH_2\underline{C}H_2CONH(\underline{C}H_2)_2$), 21.35 (t, 3-$CH_2$).

Analysis calculated for $C_{38}H_{38}N_4O_2S$ requires:
C, 74.2; H, 6.2; N, 9.1; S, 5.2%.
Found: C, 74.4; H, 6.4; N, 9.0; S, 5.2.%

Elution with EtOAc:light petroleum (2:3) gave 2,2'-dithiobis[*N*-(2-phenylethyl)-3-(3-indolyl)-propanamide] (**54**) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONH(CH_2)_2Ph$] (0.36 g, 61%) as an oil.

^{1}H NMR ($CDCl_3$): δ 8.42 (1H, s, NH), 7.51 (1H, d, J = 8.0 Hz, ArH), 7.32-7.16 (5H, m, ArH), 7.04 (3H, m, ArH), 4.63 (1H, t, J = 5.9 Hz, $N\underline{H}CH_2$), 3.23 (2H, q, J = 6.7 Hz, $NHC\underline{H}_2$, 2.85 (t, J = 7.8 Hz, 3-CH_2), 2.59 (2H, t, J = 7.0 Hz, $NHCH_2C\underline{H}_2$), 1.81 (2H, t, J = 7.8 Hz, 3-$CH_2C\underline{H}_2$).

^{13}C NMR: δ 171.95 (s, CONH), 139.15, 137.23 (2xs, Ar), 128.87, 128.55 (2xd, 2x2C, Ar), 127.02 (s, Ar), 126.39 (d, Ar), 125.50 (s, Ar), 124.33 (d, Ar), 123.98 (s, Ar), 120.11, 119.88, 111.17 (3xd, Ar), 40.62, 37.37, 35.58 (3xt, 3-$CH_2\underline{H}_2CONH(\underline{C}H_2)_2$), 20.64 (t, 3-$CH_2$).

HRFABMS m/z calculated for $C_{38}H_{39}N_4O_2S_2$:

647.2514 (MH^+)

Found: 647.2471.

Compounds 55 and 56 of Table 1

A solution of 3-(3-indolyl)propanoic acid [II: R_1 = R_3 = H, R_2 = $(CH_2)_2COOH$] (0.80 g), triethylamine (10 mL) and methyl 4-(aminomethyl)benzoate hydrochloride (Nair MG, Baugh CM, J. Org. Chem. 1973;38:2185) (1.29 g) in THF (20 mL) was stirred at 20°C for 15 minutes, then cooled to 0°C. DEPC (98%, 1.00 mL) was added, then the mixture was stirred at 20°C for 18 hours. Workup and chromatography on silica gel, eluting with EtOAc:light petroleum (5:3) gave *N*-(4-methoxycarbonylbenzyl)-3-(3-indolyl)propanamide [II: R_1 = R_3 = H, R_2 = $(CH_2)_2CONHCH_2Ph\{4\text{-}COOMe\}$] (1.10 g, 77%); mp ($CH_2Cl_2$/light petroleum) 130-132°C.

^{1}H NMR ($CDCl_3$): δ 8.08 (1H, s, NH), 7.88 (2H, d, J = 8.2 Hz, ArH), 7.60 (1H, d, J = 7.8 Hz, ArH), 7.36 (1H, d, J = 8.1 Hz, ArH), 7.19 (1H, ddd, J = 8.1, 7.1, 0.9 Hz, ArH), 7.11 (1H, ddd, J = 7.9, 7.2, 0.7 Hz, ArH), 7.06 (2H, d, J = 8.2 Hz, ArH), 6.94 (1H, d, J = 2.3 Hz, H-2), 5.74 (1H, br t, J = 5.9 Hz, $N\underline{H}CH_2$),

4.38 (2H, d, J = 5.9 Hz, $NHC\underline{H}_2$), 3.90 (3H, s, OCH_3), 3.15, 2.63 (2x2H, 2xt, J = 7.2 Hz, 3-CH_2CH_2).
^{13}C NMR: δ 172.68 (s, CONH), 166.87 (s, $\underline{C}OOCH_3$), 143.50, 136.37 (2xs, Ar), 129.80 (2xd, Ar), 129.10 (s, Ar), 127.28 (2xd, Ar), 127.03 (s, Ar), 122.11, 121.92, 119.41, 118.64 (4xd, Ar), 114.66 (s, Ar), 111.27 (d, Ar), 52.09 (q, OCH_3), 43.05 (t, $NHCH_2$), 37.37 (t, 3-$CH_2\underline{C}H_2$), 21.39 (t, 3-CH_2).
Analysis calculated for $C_{20}H_{20}N_2O_3$ requires:
C, 71.4; H, 6.0; N, 8.3%.
Found: C, 71.1; H, 5.7; N, 8.4%.

The above methoxycarbonylbenzylpropanamide (1.08 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with EtOAc:light petroleum (2:3), to give firstly 2,2'-thiobis[*N*-(4-methoxycarbonylbenzyl)-3-(3-indolyl)propanamide] [VI: n = 1; R_1 = R_3 = H, R_2 = $(CH_2)_2CONHCH_2Ph\{4\text{-}COOMe\}$] (0.18 g, 16%); mp (MeOH/dilute HCl) 101-104.5°C (dec).
^1H NMR ($CDCl_3$): δ 10.28 (1H, s, NH), 7.47 (1H, d, J = 7.7 Hz, ArH), 7.45 (2H, d, J = 8.4 Hz, ArH), 7.05 (1H, d, J = 8.0 Hz, ArH), 6.97 (1H, ddd, J = 8.0, 6.9, 1.1 Hz, ArH), 6.91 (1H, ddd, J = 7.9, 6.8, 1.1 Hz, ArH), 6.61 (2H, d, J = 8.3 Hz, ArH), 6.34 (1H, br t, J = 5.8 Hz, $N\underline{H}CH_2$), 4.40 (2H, d, J = 5.9 Hz, $NHC\underline{H}_2$), 3.79 (3H, s, OCH_3) 3.54, 2.97 (2x2H, 2xm, 3-CH_2CH_2).
^{13}C NMR: δ 174.37 (s, CONH), 166.75 (s, $\underline{C}OOCH_3$), 142.31, 137.15 (2xs, Ar), 129.35 (d, 2C, Ar), 128.39, 126.52 (2xs, Ar), 126.24 (d, 2C, Ar), 125.30 (s, Ar), 122.65, 118.87, 118.49 (3xd, Ar), 117.92 (s, Ar), 111.31 (d, Ar), 51.95 (q, OCH_3), 43.22 (t, $NHCH_2$), 36.34 (t, 3-$CH_2\underline{C}H_2$), 21.17 (t, 3-CH_2).

Analysis calculated for $C_{40}H_{38}N_4O_6S \cdot 0.5H_2O$ requires:
C, 67.5; H, 5.5; N, 7.9%.
Found: C, 67.4; H, 5.4; N, 8.1%.

Elution with EtOAc:light petroleum (1:1) gave 2,2'-dithiobis[*N*-(4-methoxycarbonylbenzyl)-3-(3-indolyl)propanamide] (**55**) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHCH_2Ph\{4\text{-}COOMe\}$] (0.50 g, 42%); mp (EtOAc/light petroleum) 151-153°C.
^1H NMR ($(CD_3)_2SO$): δ 11.42 (1H, s, NH), 8.06 (1H, t, *J* = 5.7 Hz, $N\underline{H}CH_2$), 7.81 (2H, d, *J* = 8.2 Hz, ArH), 7.55 (1H, d, *J* = 8.0 Hz, ArH), 7.34 (1H, d, *J* = 8.2 Hz, ArH), 7.17 (1H, t, *J* = 7.6 Hz, ArH), 7.11 (2H, d, *J* = 8.1 Hz, ArH), 6.99 (1H, t, *J* = 7.5 Hz, ArH), 4.19 (2H, d, *J* = 5.8 Hz, $NHC\underline{H}_2$), 3.84 (3H, s, OCH_3), 2.73, 2.24 (2x2H, 2xt, *J* = 7.5 Hz, $3\text{-}CH_2\underline{C}H_2$).
^{13}C NMR: δ 171.48 (s, CONH), 166.00 (s, $\underline{C}OOCH_3$), 145.01, 137.37 (2xs, Ar), 128.98 (d, 2C, Ar), 127.84 (s, Ar), 127.01 (d, 2C, Ar), 126.53, 125.21 (2xs, Ar), 123.24 (d, Ar), 122.39 (s, Ar), 119.57, 118.86, 111.38 (3xd, Ar), 51.93 (q, OCH_3), 41.62 (t, $NHCH_2$), 36.65 (t, $3\text{-}CH_2\underline{C}H_2$), 20.38 (t, $3\text{-}CH_2$).
Analysis calculated for $C_{40}H_{38}N_4O_6S_2$ requires:
C, 65.4; H, 5.2; N, 7.6; S, 8.7%.
Found: C, 65.5; H, 5.5; N, 7.3; S, 8.8%.

Hydrolysis of **55** (0.24 g) with K_2CO_3 in MeOH/water at 30°C for 1 day, then 50°C for 1 hour, under nitrogen as above gave an oil. Chromatography on silica gel, eluting with EtOAc:light petroleum (1:1) containing 1% AcOH, gave 2,2'-dithiobis[*N*-(4-carboxybenzyl)-3-(3-indolyl)propanamide] (**56**) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHCH_2Ph\{4\text{-}COOH\}$] (60 mg, 26%); mp (MeOH/dilute HCl) 135.5-138.5°C (decomposed).
^1H NMR ($(CD_3)_2SO$): δ 11.41 (1H, s, NH), 8.03 (1H, t, *J* = 5.8 Hz, $N\underline{H}CH_2$), 7.79 (2H, d, *J* = 8.2 Hz, ArH), 7.55

(1H, d, J = 8.0 Hz, ArH), 7.33 (1H, d, J = 8.2 Hz, ArH), 7.16 (1H, t, J = 7.6 Hz, ArH), 7.09 (2H, d, J = 8.1 Hz, ArH), 6.99 (1H, t, J = 7.5 Hz, ArH), 4.18 (2H, d, J = 5.8 Hz, $NHC\underline{H}_2$), 2.73, 2.23 (2x2H, 2xt, J = 7.5 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 171.44 (s, CONH), 167.10 (s, COOH), 144.46, 137.37 (2xs, Ar), 129.14 (d, 2C, Ar), 129.05 (s, Ar), 126.87 (d, 2C, Ar), 126.53, 125.18 (2xs, Ar), 123.23 (d, Ar), 122.40 (s, Ar), 119.58, 118.85, 111.37 (3xd, Ar), 41.65 (t, $NHCH_2$), 36.42 (t, 3-$CH\underline{C}H_2$), 20.37 (t, 3-CH_2).

Analysis calculated for $C_{38}H_{34}N_4O_6S_2 \cdot H_2O$ requires:
C, 63.0; H, 5.0; N, 7.7; S, 8.8%.
Found: C, 62.5; H, 5.2; N, 8.2; S, 8.8%.

Compounds 57 and 58 of Table 1

A stirred solution of methyl 2-acetoxy-4-bromomethylbenzoate (Regnier G, Canevari R, Le Douarec J-C, Bull. Soc. Chim. Fr. 1966:2821) (10.7 g) and hexamethylenetetramine (17.1 g) in $CHCl_3$ (150 mL) was refluxed for 5 hours, then the solvent was removed (method of Meindl W, v Angerer E, Ruckdeschel G, Schonenberger H, Arch. Pharm. (Weinheim) 1982;315:941). The residue was stirred with MeOH (60 mL) and concentrated HCl (30 mL) at 20°C for 10 minutes, then the solvent removed. Treatment of the solid residue twice more with HCl/MeOH and evaporation gave a solid, which was washed with CH_2Cl_2, then treated with saturated $KHCO_3$ solution. The base was extracted with EtOAc and CH_2Cl_2, then the solvents removed. The crude hydrochloride salt (5.30 g, 70% pure) was precipitated from an ethereal solution of the base upon the addition of HCl gas. A subsample of the

above crude base was purified by chromatography on silica gel, eluting with EtOAc/light petroleum (1:2). Acidification of a solution of the purified base gave pure methyl 4-(aminomethyl)-2-hydroxybenzoate hydrochloride; mp (CH_2Cl_2/light petroleum) 225-227°C.
^{1}H NMR $(CD_3)_2SO$): δ 10.56 (1H, s, OH), 8.58 (3H, br s, NH_3^+), 7.78 (1H, d, J = 8.1 Hz, H-6), 7.14 (1H, s, H-3), 7.05 (1H, d, J = 8.1 Hz, H-5), 4.01 (2H, br s, 4-CH_2), 3.88 (3H, s, OCH_3).
^{13}C NMR: δ 168.81 (s, $\underline{C}OOCH_3$), 159.80 (s, C-2), 141.84 (s, C-4), 130.25 (d, C-6), 119.61 (d, C-5), 117.48 (d, C-3), 112.90 (s, C-1), 52.53 (q, OCH_3), 41.63 (t, 4-CH_2).
Analysis calculated for $C_9H_{11}NO_3 \cdot HCl \cdot 0.5H_2O$ requires:
C, 47.7; H, 5.8; N, 6.2; Cl, 15.7%.
Found: C, 47.9; H, 5.8; N, 6.3; Cl, 15.9%.

A solution of 3-(3-indolyl)propanoic acid [II: R_1 = R_3 = H, R_2 = $(CH_2)_2COOH$] (1.50 g), triethylamine (10 mL) and crude methyl 4-(aminomethyl)-2-hydroxybenzoate hydrochloride (3.46 g) in DMF (20 mL) was stirred at 20°C for 10 minutes, then cooled to 0°C. DEPC (98%, 1.47 mL) was added, then the mixture was stirred at 20°C for 17 hours. Workup and chromatography on silica gel, eluting with EtOAc:light petroleum (1:1) gave *N*-(3-hydroxy-4-methoxycarbonyl-benzyl)-3-(3-indolyl)propanamide [II: R_1 = R_3 = H, R_2 = $(CH_2)_2CONHCH_2Ph${3-OH, 4-COOMe}] (1.40 g, 50%); mp (EtOAc/light petroleum) 132-133°C.
^{1}H NMR ($(CD_3)_2SO$): δ 10.76 (1H, br s, NH), 10.50 (1H, s, OH), 8.41 (1H, t, J = 5.8 Hz, $N\underline{H}CH_2$), 7.70 (1H, d, J = 8.1 Hz, ArH), 7.54 (1H, d, J = 7.8 Hz, ArH), 7.33 (1H, d, J = 8.1 Hz, ArH), 7.10 (1H, d, J = 2.2 Hz, H-2), 7.06 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 6.97 (1H, ddd, J = 7.8, 7.0, 0.8 Hz, ArH), 6.83 (1H, d,

J = 1.4 Hz, ArH), 6.74 (1H, dd, J = 8.2, 1.4 Hz, ArH), 4.27 (2H, d, J = 6.0 Hz, $NHC\underline{H}_2$), 3.88 (3H, s, OCH_3), 2.96, 2.54 (2x2H, 2xt, J = 7.7 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 172.05 (s, CONH), 169.14 (s, $\underline{C}OOCH_3$), 160.10, 148.27, 136.22 (3xs, Ar), 129.92 (d, Ar), 126.98 (s, Ar), 122.14, 120.84, 118.30, 118.12, 118.09, 115.41 (6xd, Ar), 113.68 (s, Ar), 111.27 (d, Ar), 111.20 (s, Ar), 52.34 (q, OCH_3), 41.67 (t, $NHCH_2$), 36.23 (t, 3-$CH_2\underline{C}H_2$), 21.00 (t, 3-CH_2).

Analysis calculated for $C_{20}H_{20}N_2O_4$ requires:
C, 68.2; H, 5.7; N, 8.0%.
Found: C, 68.3; H, 5.9; N, 8.0%.

A solution of acetyl chloride (0.42 mL) in THF (5 mL) was added to a stirred solution of the above propanamide (1.22 g) and triethylamine (1.00 mL) in THF (15 mL) at 0°C, then the mixture was stirred at 20°C for 18 hours. The reaction was then quenched with water (100 mL) and extracted with EtOAc (3 x 100 mL). Evaporation and chromatography on silica gel, eluting with EtOAc:light petroleum (2:1) gave *N*-(3-acetoxy-4-methoxycarbonylbenzyl)-3-(3-indolyl)propanamide [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHCH_2Ph\{3\text{-}OAc, 4\text{-}COOMe\}$] (1.28 g, 94%) as an oil.

^1H NMR ($CDCl_3$): δ 8.18 (1H, br s, NH), 7.87 (1H, d, J = 8.1 Hz, ArH), 7.57 (1H, d, J = 8.0 Hz, ArH), 7.31 (1H, dt, J = 8.1, 0.8 Hz, ArH), 7.17 (1H, ddd, J = 8.1, 7.0, 1.1 Hz, ArH), 7.09 (1H, ddd, J = 7.9, 7.0, 0.9 Hz, ArH), 6.97 (1H, dd, J = 8.1, 1.6 Hz, ArH), 6.84 (1H, d, J = 1.5 Hz, ArH), 6.77 (1H, d, J = 2.3 Hz, H-2), 5.67 (1H, br t, J = 5.8 Hz, $N\underline{H}CH_2$), 4.31 (2H, d, J = 6.0 Hz, $NHC\underline{H}_2$), 3.87 (3H, s, $COOCH_3$), 3.11, 2.58 (2x2H, 2xt, J = 6.9 Hz, 3-CH_2CH_2), 2.36 (3H, s, $OCOCH_3$).

^{13}C NMR: δ 172.84 (s, CONH), 170.14 (s, $O\underline{C}OCH_3$), 164.64 (s, $\underline{C}OOCH_3$), 150.82, 145.26, 136.33 (3xs, Ar),

132.04 (d, Ar), 126.85 (s, Ar), 125.42, 122.93, 122.31, 121.95 (4xd, Ar), 121.87 (s, Ar), 119.28, 118.52 (2xd, Ar), 114.08 (s, Ar), 111.36 (d, Ar), 52.23 (q, OCH_3), 42.62 (t, $NHCH_2$), 37.32 (t, 3-$CH_2\underline{C}H_2$), 21.46 (t, 3-CH_2), 21.06 (q, $OCO\underline{C}H_3$).

HREIMS *m/z* calculated for $C_{22}H_{22}N_2O_5$:

394.1529 (M^+).

Found: 394.1526.

The above *O*-acetate (1.47 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. Hydrolysis of the resulting oil with excess $KHCO_3$ in MeOH/water at 20°C for 1 hour (to remove the acetate group) gave an oil which was purified by chromatography on silica gel. Elution with EtOAc:light petroleum (1:2) gave firstly 2,2'-thiobis[*N*-(3-hydroxy-4-methoxycarbonylbenzyl)-3-(3-indolyl)propanamide] [VI: n = 1; R_1 = R_3 = H, R_2 = $(CH_2)_2CONHCH_2Ph\{3\text{-}OAc, 4\text{-}COOMe\}$] (0.12 g, 9%); mp (MeOH/dilute HCl) 109-112°C (decomposed).

^1H NMR ($CDCl_3$): δ 10.50 (1H, s, OH), 10.17 (1H, s, NH), 7.49 (1H, d, *J* = 7.9 Hz, ArH), 7.31 (1H, d, *J* = 8.2 Hz, ArH), 7.19 (1H, d, *J* = 8.1 Hz, ArH), 7.07 (1H, ddd, *J* = 8.0, 7.1, 0.8 Hz, ArH), 6.97 (1H, ddd, *J* = 7.8, 7.2, 0.6 Hz, ArH), 6.32 (1H, d, *J* = 1.1 Hz, ArH), 5.98 (1H, dd, *J* = 8.2, 1.5 Hz, ArH), 5.72 (1H, t, *J* = 5.7 Hz, $N\underline{H}CH_2$), 4.22 (2H, d, *J* = 5.7 Hz, $NHC\underline{H}_2$), 3.86 (3H, s, OCH_3), 3.50, 2.88 (2x2H, 2xm, 3-CH_2CH_2).

^{13}C NMR: δ 173.77 (s, CONH), 170.06 (s, $\underline{C}OOCH_3$), 161.36, 145.57, 137.16 (3xs, Ar), 130.02 (d, Ar), 126.62, 125.16 (2xs, Ar), 122.69, 119.13, 118.43 (3xd, Ar), 117.65 (s, Ar), 117.40, 115.51, 111.53 (3xd, Ar), 111.07 (s, Ar), 52.18 (q, OCH_3), 43.19 (t, $NHCH_2$), 36.32 (t, 3-$CH_2\underline{C}H_2$), 21.22 (t, 3-CH_2).

Analysis calculated for $C_{40}H_{38}N_4O_8S$ requires:
C, 65.4; H, 5.2; N, 7.6; S, 4.4%.
Found: C, 65.2; H, 5.1; N, 7.4; S, 4.4%.

Elution with EtOAc:light petroleum (2:3) gave 2,2'-dithiobis[*N*-(3-hydroxy-4-methoxycarbonylbenzyl)-3-(3-indolyl)propanamide] (**57**) [V: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHCH_2Ph\{3\text{-}OH, 4\text{-}COOMe\}$] (0.38 g, 27%); mp (MeOH) 183-185°C.

^1H NMR ($CDCl_3$): δ 10.80 (1H, s, OH), 8.65 (1H, s, NH), 7.67 (1H, d, J = 8.1 Hz, ArH), 7.52 (1H, d, J = 8.0 Hz, ArH), 7.27 (1H, d, J = 7.7 Hz, ArH), 7.15 (1H, ddd, J = 8.1, 7.2, 0.9 Hz, ArH), 7.01 (1H, ddd, J = 7.9, 7.2, 0.7 Hz, ArH), 6.55 (1H, d, J = 1.5 Hz, ArH), 6.52 (1H, dd, J = 8.2, 1.5 Hz, ArH), 5.10 (1H, t, J = 5.9 Hz, $N\underline{H}CH_2$), 4.13 (2H, d, J = 6.0 Hz, $NHC\underline{H}_2$), 3.94 (3H, s, OCH_3), 2.88, 1.94 (2x2H, 2xt, J = 7.7 Hz, 3-$CH_2\underline{C}H_2$).

^{13}C NMR: δ 172.12 (s, CONH), 170.39 (s, $\underline{C}OOCH_3$), 161.55, 146.95, 137.29 (3xs, Ar), 130.09 (d, Ar), 127.01, 125.87 (2xs, Ar), 124.39 (d, Ar), 123.79 (s, Ar), 120.16, 119.86, 118.34, 115.69, 111.37 (5xd, Ar), 111.20 (s, Ar), 52.31 (q, OCH_3), 42.82 (t, $NHCH_2$), 37.09 (t, 3-$CH_2\underline{C}H_2$), 20.54 (t, 3-CH_2).

Analysis calculated for $C_{40}H_{38}N_4O_8S_2$ requires:
C, 62.7; H, 5.0; N, 7.3; S, 8.4%.
Found: C, 62.5; H, 4.9; N, 7.3; S, 8.4%.

Hydrolysis of **57** (0.28 g) with K_2CO_3 in MeOH/water at 50°C for 5 hours, under nitrogen as above, gave an oil. Chromatography on silica gel, eluting with EtOAc:light petroleum (1:1) containing 1% AcOH, gave 2,2'-dithiobis[*N*-(4-carboxy-3-hydroxybenzyl)-3-(3-indolyl)propanamide] (**58**) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2CONHCH_2Ph\{3\text{-}OH, 4\text{-}COOH\}$] (72 mg, 27%); mp (MeOH/dilute HCl) 160-163.5°C (dec).

^{1}H NMR $(CD_3)_2SO$): δ 11.39 (1H, s, NH), 8.03 (1H, t, J = 5.9 Hz, N<u>H</u>CH_2), 7.65 (1H, d, J = 8.1 Hz, ArH), 7.54 (1H, d, J = 8.0 Hz, ArH), 7.32 (1H, d, J = 8.2 Hz, ArH), 7.16 (1H, ddd, J = 8.1, 7.1, 1.0 Hz, ArH), 6.99 (1H, ddd, J = 7.8, 7.1, 0.7 Hz, ArH), 6.72 (1H, d, J = 1.3 Hz, ArH), 6.57 (1H, dd, J = 8.2, 1.4 Hz, ArH), 4.13 (2H, d, J = 5.9 Hz, NHC<u>H</u>$_2$), 2.75, 2.24 (2x2H, 2xt, J = 7.8 Hz, 3-CH_2CH_2).

^{13}C NMR: δ 171.70 (s, CONH), 171.47 (s, COOH), 161.04, 147.83, 137.37 (3xs, Ar), 130.08 (d, Ar), 126.51, 125.11 (2xs, Ar), 123.25 (d, Ar), 122.42 (s, Ar), 119.49, 118.86, 117.73, 115.09, 111.41 (5xd, Ar), 111.21 (s, Ar), 41.67 (t, $NHCH_2$), 36.63 (t, 3-CH_2<u>CH_2</u>), 20.41 (t, 3-CH_2).

Analysis calculated for $C_{38}H_{34}N_4O_8S_2 \cdot H_2O$ requires:
C, 60.3; H, 4.8; N, 7.4; S, 8.5%.
Found: C, 60.2; H, 4.9; N, 7.1; S, 8.5%.

<u>Compound 59 of Table 1</u>

3-(3-Indolyl)propanoic acid [II: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (0.95 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above, to give crude 2,2'-dithiobis[3-(3-indolyl)propanoic acid] [VI: n = 2; $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOH$] (1.12 g) as an oil. DEPC (98%, 1.00 mL) was added to a stirred solution of this oil, triethylamine (0.84 mL) and aniline (1.55 mL) in THF (15 mL) at 0°C, then the mixture was stirred at 20°C for 1 day. Dilute KOH (0.1 M, 100 mL) was added and the mixture stirred for 30 minutes (in an attempt to cleave the DEPC adduct and reform the disulfide), then the mixture extracted with CH_2Cl_2 (3 x 100 mL). Evaporation gave an oil which was partly purified by

chromatography on silica gel, eluting with EtOAc/light petroleum (2:1). The yellow disulfide was further purified by chromatography on fresh silica gel, eluting with CH_2Cl_2, then $CHCl_3$:EtOH (99:1), to give 2,2'-dithiobis[*N*-phenyl-3-(3-indolyl)propanamide] (59) [VI: n = 2; R_1 = R_3 = H, R_2 = $(CH_2)_2CONHPh$] (0.23 g, 16% overall); mp (CH_2Cl_2/benzene) 181-182.5°C (an analytical sample recrystallized from CH_2Cl_2/light petroleum decomposed above 114°C).

^{1}H NMR ($(CD_3)_2CO$): δ 10.52 (1H, s, NH), 8.88 (1H, s, NHPh), 7.64 (1H, d, J = 8.0 Hz, ArH), 7.56 (2H, dd, J = 7.5, 0.9 Hz, ArH), 7.37 (1H, d, J = 8.2 Hz, ArH), 7.24 (2H, dd, J = 8.4, 7.5 Hz, ArH(Ph)), 7.16 (1H, ddd, J = 8.1, 7.1, 1.1 Hz, ArH), 7.02 (2H, m, ArH), 3.04, 2.54 (2x2H, 2xm, 3-CH_2CH_2).

^{13}C NMR: δ 171.48 (s, CONH), 140.24, 138.80 (2xs, Ar), 129.37 (2xd, Ar), 128.17, 126.81 (2xs, Ar) 124.57, 124.02 (2xd, Ar), 123.86 (s, Ar), 120.62, 120.36 (2xd, Ar), 120.23 (2xbr d, Ar), 112.38 (d, Ar), 38.97 (t,3-$CH_2\underline{C}H_2$) 21.39 (t, 3-CH_2).

Analysis calculated for $C_{34}H_{30}N_4O_2S_2 \cdot 0.5H_2O$ requires:
C, 68.1; H, 5.2; N, 9.4; S, 10.7%.
Found: C, 68.3; H, 5.1; N, 9.3; S, 10.9%.

Compound 60 of Table 1

DEPC (98%, 0.72 mL) was added to a stirred solution of DL-*N*-acetyltryptophan (1.00 g) and benzylamine (2.0 mL) in DMF (10 mL) at 0°C, then the mixture was stirred at 20°C for 16 hours. The reaction was then quenched with water and extracted with EtOAc. Evaporation gave an oil which was chromatographed on silica gel. Elution with CH_2Cl_2 and EtOAc gave firstly foreruns, then DL-α-acetylamino-*N*-benzyl-3-(3-indolyl)-propanamide [II: R_1 = R_3 = H,

R_2 = $CH_2CH(NHAc)CONHCH_2Ph$] (0.82 g, 60%);
mp (CH_2Cl_2/light petroleum) 169-170°C.
^1H NMR ($(CD_3)_2SO$): δ 10.80 (1H, s, NH), 8.47 (1H, br t, J = 5.8 Hz, $N\underline{H}CH_2$), 8.08 (1H, d, J = 8.1 Hz, $CHN\underline{H}$), 7.61 (1H, d, J = 7.8 Hz, ArH), 7.33 (1H, d, J = 8.1 Hz, ArH), 7.26 (2H, dt, J = 7.1, 1.5 Hz, ArH), 7.20 (1H, dt, J = 7.2, 1.5 Hz, ArH), 7.13 (1H, m, H-2), 7.12 (2H, d, J = 7.2 Hz, ArH), 7.06 (1H, ddd, J = 7.9, 7.1, 0.9 Hz, ArH), 6.97 (1H, ddd, J = 7.9, 7.0, 0.9 Hz, ArH), 4.57 (1H, td, J = 8.3, 5.7 Hz, 3-$CH_2C\underline{H}$), 4.28, 4.24 (2x1H, 2xdd, J = 15.9, 5.9 Hz, $NHC\underline{H}_2$), 3.13 (1H, dd, J = 14.4, 5.6 Hz, 3-CH), 2.93 (1H, dd, J = 14.4, 8.6 Hz, 3-CH), 1.80 (3H, s, $COCH_3$).
^{13}C NMR: δ 171.59 (s, $COCH_3$), 169.02 (s, CONH), 139.18, 135.99 (2xs, Ar), 128.06 (d, 2C, Ar), 127.21 (s, Ar), 126.87 (d, 2C, Ar), 126.49, 123.47, 120.75, 118.39, 118.10, 111.17 (6xd, Ar), 110.11 (s, Ar), 53.53 (d, CH), 41.91 (t, $NHCH_2$), 27.92 (t, 3-CH_2), 22.50 (q, CH_3).
Analysis calculated for $C_{20}H_{21}N_3O_2$ requires:
C, 71.6; H, 6.3; N, 12.5%.
Found: C, 71.5; H, 6.4; N, 12.6%.

Acidification of the aqueous portion with dilute HCl, extraction with EtOAc and evaporation gave *N*-acetyltryptophan (0.30 g, 30%); mp (EtOAc/light petroleum) 204-206°C.

The above α-acetamide (1.25 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was treated successively with $NaBH_4$ then H_2O_2 as described above. The resulting oil was chromatographed on silica gel, eluting with CH_2Cl_2:EtOAc (2:1) to give firstly 2,2'-thiobis[α-acetylamino-*N*-benzyl-3-(3-indolyl)propanamide] [VI: n = 1; R_1 = R_3 = H, R_2 = $CH_2CH(NHAc)CONHCH_2Ph$] (0.30 g, 23%) as a mixture

of diastereoisomers; mp (EtOAc/light petroleum) 190-194°C.

^{1}H NMR ($(CD_3)_2SO$): δ 10.97, 10.94 (2x1H, 2xs, NH), 8.50, 8.48 (2x1H, 2xbr t, J = 5.8 Hz, $N\underline{H}CH_2$), 8.17, 8.15 (2x1H, d, J = 8.4 Hz, $CHN\underline{H}$), 7.63 (2x1H, d, J = 7.7 Hz, ArH), 7.3-6.9 (2x8H, m, ArH), 4.75 (2x1H, m, 3-$CH_2C\underline{H}$), 4.27, 4.19 (4x1H, 2xdd, J = 16.1, 5.7 Hz, $NHC\underline{H}_2$), 3.44 (2x1H, m, 3-CH), 3.18 (2x1H, m, 3-CH), 1.79 (2x3H, 2xs, $COCH_3$).

^{13}C NMR: δ 171.20, 171.18 (2xs, $\underline{C}OCH_3$), 169.13 (s, 2C, CONH), 138.83, 138.79 (2xs, Ar), 136.66 (s, 2C, Ar), 128.03, 128.01 (2xd, 2x2C, Ar), 127.42 (s, 2C, Ar), 126.96, 126.91 (2d, 2x2C, Ar), 126.51, 126.48 (2xd, Ar), 124.58, 124.55 (2xs, Ar), 121.97 (d, 2x2C, Ar), 119.02, 118.98 (2xd, Ar), 118.66 (d, 2C, Ar), 115.01, 114.94 (2xs, Ar), 110.79 (d, 2C, Ar), 53.66, 53.59 (2xd, 3-$CH_2\underline{C}H$), 42.13 (t, 2C, $NHCH_2$), 28.14, 28.07 (2xt, 3-CH_2), 22.52 (q, 2C, CH_3).

Analysis calculated for $C_{40}H_{40}N_6O_4S\cdot0.5H_2O$ requires:
C, 67.7; H, 5.8; N, 11.9; S, 4.5%.
Found: C, 67.7; H, 5.8; N, 11.9; S, 5.1%.

Elution with CH_2Cl_2:EtOAc (1:2) gave 2,2'-dithiobis[α-acetylamino-*N*-benzyl-3-(3-indolyl)-propanamide] (**60**) [VI: n = 2; R_1 = R_3 = H, R_2 = $CH_2CH(NHAc)CONHCH_2Ph$] (0.84 g, 62%) as a yellow oil (a mixture of diastereoisomers). Crystallizations from CH_2Cl_2/light petroleum gave a single pair of diastereoisomers; mp 140-144°C (dec).

^{1}H NMR ($CDCl_3$): δ 9.16 (1H, s, NH), 7.51 (1H, d, J = 8.1 Hz, ArH), 7.2-7.0 (6H, m, ArH), 6.89 (2H, m, ArH), 6.76 (1H, d, J = 7.2 Hz, $CHN\underline{H}$), 6.16 (1H, t, J = 5.8 Hz, $N\underline{H}CH_2$), 4.64 (1H, q, J = 7.2 Hz, 3-$CH_2C\underline{H}$), 4.20, 4.12 (2x1H, 2xdd, J = 14.8, 5.9 Hz, $NHC\underline{H}_2$), 3.13

(1H, dd, J = 14.0, 7.1 Hz, 3-CH), 2.96 (1H, dd, J = 14.0, 7.3 Hz, 3-CH), 1.84 (3H, s, $COCH_3$).

Analysis calculated for $C_{40}H_{40}N_6O_4S_2 \cdot 0.5H_2O$ requires:

C, 64.8; H, 5.5; N, 11.3; S, 8.6 %.

Found: C, 65.0; H, 5.4; N, 11.3; S, 8.8%.

Crystallizations from EtOAc/light petroleum gave the other pair of diastereoisomers of **60**; mp 154.5-157.5°C (dec).

^1H NMR ($CDCl_3$): δ 9.27 (1H, s, NH), 7.42 (1H, d, J = 8.0 Hz, ArH), 7.28-7.12 (6H, m, ArH), 7.04 (1H, dd, J = 7.8, 7.0 Hz, ArH), 6.75 (2H, m, ArH), 6.45 (1H, br d, J = 7.1 Hz, CHNH), 5.90 (1H, br s, $N\underline{H}CH_2$), 4.41 (1H, q, J = 7.4 Hz, 3-$CH_2C\underline{H}$), 4.17 (1H, dd, J = 14.8, 6.0 Hz, NHC<u>H</u>), 4.08 (1H, dd, J =14.8, 5.0 Hz, NHC<u>H</u>), 2.99 (1H, dd, J = 14.0, 6.9 Hz, 3-CH), 2.93 (1H, dd, J = 13.9, 7.6 Hz, 3-CH), 1.82 (3H, s, $COCH_3$).

^{13}C NMR: δ 170.74 (s, $\underline{C}OCH_3$), 169.92 (s, CONH), 137.42, 137.28 (2xs, Ar), 128.58 (d, 2C, Ar), 127.59 (s, Ar), 127.51 (d, 2C, Ar), 127.40 (d, Ar), 126.26 (s, Ar), 124.39, 120.37, 119.51 (3xd, Ar), 118.96 (s, Ar), 111.51 (d, Ar), 54.63 (d, 3-$CH_2\underline{C}H$), 43.70 (t, $NHCH_2$), 28.87 (t, 3-CH_2), 23.23 (q, CH_3).

Analysis calculated for $C_{40}H_{40}N_6O_4S_2$ requires:

C, 65.6; H, 5.5; N, 11.5; S, 8.7%.

Found: C, 65.4; H, 5.6; N, 11.5; S, 8.7%.

In DMSO solution, both pure diastereomers reverted to a 1:1 mixture of diastereoisomers by disulfide exchange within 3 minutes.

<u>Compounds 61 and 62 of Table 1</u>

Ethyl trifluoroacetate (1.7 mL) was added to a stirred solution of DL-tryptophan (2.3 g) and triethylamine (1.6 mL) in DMF (5 mL), then the flask was sealed and purged with nitrogen, and the mixture

stirred at 20°C for 1 day (method of Curphey TJ, J. Org. Chem. 1979;44:2805). Excess reagents were removed under vacuum, then triethylamine (1.9 mL) and DMF (10 mL) were added, and the mixture cooled to 0°C. DEPC (98%, 2.0 mL) was added, followed by benzylamine (1.72 mL), then the mixture was stirred under nitrogen at 20°C for 1 day. The resulting solution was diluted with water (100 mL) and extracted with EtOAc (3 x 100 mL). Evaporation gave an oil which was purified by chromatography on silica gel, eluting with EtOAc:light petroleum (1:1), to give DL-*N*-benzyl-α-trifluoroacetylamino-3-(3-indolyl)propanamide [II: $R_1 = R_3 = H$, $R_2 = CH_2CH(NHCOCF_3)CONHCH_2Ph$] (2.21 g, 50%); mp (EtOAc/light petroleum) 181-183°C.

^1H NMR ($(CD_3)_2SO$): δ 10.84 (1H, s, NH), 9.65 (1H, br s, CHNH), 8.79 (1H, t, J = 5.5 Hz, $NHCH_2$), 7.67 (1H, d, J = 7.8 Hz, ArH), 7.34 (1H, d, J = 8.0 Hz, ArH), 7.30 (2H, t, J = 7.2 Hz, ArH), 7.23 (1H, t, J = 7.3 Hz, ArH), 7.18 (2H, d, J = 7.5 Hz, ArH), 7.15 (1H, d, J = 2.2 Hz, H-2), 7.07 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 6.98 (1H, dd, J = 7.8, 7.0 Hz, ArH), 4.63 (1H, br m, $3\text{-}CH_2CH$), 4.32 (2H, d, J = 5.8 Hz, $NHCH_2$), 3.25 (1H, dd, J = 14.5, 5.0 Hz, 3-CH), 3.12 (1H, dd, J = 14.5, 9.9 Hz, 3-CH).

^{13}C NMR: δ 169.89 (s, CONH), 156.14, (q, J_{CF} = 36.5 Hz, $COCF_3$), 138.92, 135.97 (2xs, Ar), 128.17, 126.95 (2xd, 2x2C, Ar), 126.95 (s, Ar) 126.68, 123.77, 120.86, 118.36, 118.17 (5xd, Ar), 115.69 (q, J_{CF} = 288 Hz, CF_3), 111.24 (d, Ar), 109.41 (s, Ar), 54.24 (d, $3\text{-}CH_2CH$), 42.11 (t, $NHCH_2$), 27.08 (t, $3\text{-}CH_2$).

Analysis calculated for $C_{20}H_{18}F_3N_3O_2$ requires:
C, 61.7; H, 4.6; N, 10.8%.
Found: C, 61.9; H, 4.9; N, 10.9%.

Acidification of the aqueous portion with dilute HCl, then extraction with EtOAc (3 x 100 mL) and evaporation gave DL-α-trifluoroacetylamino-3-(3-indolyl)propanoic acid [II: $R_1 = R_3 = H$, $R_2 = CH_2CH(NHCOCF_3)COOH$] (0.72 g, 21%); mp (water) 155-157°C (Weygand F, Geiger R, Chem. Ber. 1956;89:647 record mp 162-163°C).

^1H NMR ($(CD_3)_2SO$): δ 10.86 (1H, br s, NH), 9.75 (1H, br d, J = 8.0 Hz, CHNH), 7.55 (1H, d, J = 7.8 Hz, ArH), 7.34 (1H, d, J = 8.1 Hz, ArH), 7.14 (1H, d, J = 2.3 Hz, H-2), 7.07 (1H, ddd, J = 8.0, 7.1, 0.9 Hz, ArH), 6.99 (1H, ddd, J = 7.9, 7.0, 0.9 Hz, ArH), 4.51 (1H, ddd, J = 10.2, 8.0, 4.2 Hz, 3-CH_2CH), 3.32 (1H, dd, J = 14.8, 4.3 Hz, 3-CH), 3.17 (1H, dd, J = 14.8, 10.3 Hz, 3-CH).

^{13}C NMR: δ 171.64 (s, COOH), 156.23 (q, J_{CF} = 36.5 Hz, $COCF_3$), 136.01, 126.85 (2xs, Ar), 123.45, 120.93, 118.35, 117.90 (4xd, Ar), 117.09, 115.66 (q, J_{CF} = 288 Hz, CF_3), 111.36 (d, Ar), 109.56 (s, Ar), 53.58 (d, 3-CH_2CH), 25.88 (t, 3-CH_2).

The above α-trifluoroacetamide (2.15 g) was treated with S_2Cl_2 as above, then the product mixture obtained after workup was chromatographed directly on silica gel. Elution with CH_2Cl_2 and CH_2Cl_2:EtOAc (19:1) gave foreruns, including mono- and trisulfides, then 2,2'-dithiobis[*N*-benzyl-α-trifluoroacetylamino-3-(3-indolyl)propanamide] (**61**) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = CH_2CH(NHCOCF_3)CONHCH_2Ph$] (1.01 g, 44%) as a yellow oil (a mixture of diastereoisomers). A subsample crystallized from EtOH was a single pair of diastereoisomers; mp 160-164°C (decomposed).

^1H NMR ($CDCl_3$): δ 8.76 (1H, s, NH), 7.57 (1H, d, J = 8.0 Hz, CHNH), 7.43 (1H, d, J = 7.9 Hz, ArH), 7.3-7.0 (6H, m, ArH), 6.75 (2H, m, ArH), 5.49 (1H, t,

J = 5.2 Hz, $N\underline{H}CH_2$), 4.26 (1H, td, J = 7.9, 6.4 Hz, 3-$CH_2\underline{C}H$), 4.14 (1H, dd, J = 14.8, 5.8 Hz, $NHCH_2$), 4.00 (1H, dd, J = 14.5, 4.9 Hz, $NHCH_2$) 2.99 (1H, dd, J = 14.0, 8.4 Hz, 3-CH), 2.77 (1H, dd, J = 14.0, 5.9 Hz, 3-CH).

^{13}C NMR: δ 168.87 (s, CONH), 156.81 (q, J_{CF} = 36.5 Hz, $\underline{C}OCF_3$), 137.25, 136.61 (2xs, Ar), 128.73 (d, 2C, Ar), 127.71 (d, 3C, Ar), 126.96, 126.11 (2xs, Ar), 124.97, 120.95, 119.25 (3xd, Ar), 118.14 (s, Ar), 115.62 (q, J_{CF} = 288 Hz, CF_3), 111.49 (d, Ar), 54.67 (d, 3-$CH_2\underline{C}H$), 44.02 (t, $NHCH_2$), 28.22 (t, 3-CH_2).

Analysis calculated for $C_{40}H_{34}F_6N_6O_4S_2 \cdot 0.5H_2O$ requires:
C, 56.5; H, 4.1; N, 9.9; S, 7.5%.
Found: C, 56.6; H, 4.3; N, 9.8; S, 7.6%.

The trifluoroacetamide disulfide (**61**) (0.80 g) was treated with excess $NaBH_4$ at 20°C as above, then the resulting oil was chromatographed on alumina. Elution with $CHCl_3$:EtOH (99:1) gave foreruns, then elution with $CHCl_3$:EtOH (98:2) gave 2,2'-dithiobis[α-amino-*N*-benzyl-3-(3-indolyl)propanamide] (**62**) [VI: n = 2; $R_1 = R_3 = H$, $R_2 = CH_2CH(NH_2)CONHCH_2Ph$] (0.14 g, 22%); mp ($CH_2Cl_2$/light petroleum) 147-150°C (decomposed).

^1H NMR ($(CD_3)_2SO$): δ 11.56 (1H, s, NH), 8.18 (1H, t, J = 5.8 Hz, $N\underline{H}CH_2$), 7.61 (1H, d, J = 7.8 Hz, ArH), 7.36 (1H, d, J = 8.1 Hz, ArH), 7.33-6.95 (7H, m, ArH), 4.23, 4.13 (2x1H, 2xdd, J = 15.2, 5.8 Hz, $NHC\underline{H}_2$), 3.41 (1H, br m, 3-$CH_2\underline{C}H$), 2.93 (1H, dd, J = 13.7, 4.9 Hz, 3-CH), 2.64 (1H, br m, 3-CH), 1.7 (2H, br s, NH_2).

^{13}C NMR: δ 174.12 (s, CONH), 139.13, 137.38 (2xs, Ar), 128.06, 127.02 (2xd, 2x2C, Ar), 126.95, 126.71 (2xs, Ar), 126.51, 123.19, 119.62 (3xd, Ar), 119.18 (s, Ar), 118.87, 111.39 (2xd, Ar), 55.57 (d, 3-$CH_2\underline{C}H$), 41.90 (t, $NHCH_2$), 30.58 (t, 3-CH_2).

Analysis calculated for $C_{36}H_{36}N_6O_2S_2 \cdot 0.5H_2O$ requires:
C, 65.8; H, 5.6; N, 12.8%.
Found: C, 65.8; H, 5.8; N, 12.6%.

Compound 63 of Table 1

Acetyl chloride (0.50 mL, 7.0 mmol) was added to a stirred solution of DL-3-(3-indolyl)lactic acid (1.00 g, 14.3 mmol) and Et_3N (2 mL, 14.3 mmol) in THF (5 mL) at 0°C. The mixture was stirred at 0°C for 7 hours, then at 20°C for 15 hours, quenched with water (100 mL), acidified with dilute HCl (to pH 2), then extracted with EtOAc (3 x 100 mL). Evaporation gave crude (ca. 90% pure) DL-α-acetoxy-3-(3-indolyl)-propanoic acid [II: $R_1 = R_3 = H$, $R_2 = CH_2CH(OAc)COOH$] (1.30 g) as an oil which was used directly.
^1H NMR ($(CD_3)_2SO$): δ 10.88 (1H, s, NH), 7.54 (1H, d, J = 7.8 Hz, ArH), 7.33 (1H, d, J = 8.0 Hz, ArH), 7.17 (1H, br s, H-2), 7.06 (1H, dd, J = 8.0, 7.1 Hz, ArH), 6.99 (1H, t, J = 7.4 Hz, ArH), 5.06 (1H, dd, J = 7.3, 4.9 Hz, 3-$CH_2\underline{C}H$), 3.22 (1H, dd, J = 15.1, 4.5 Hz, 3-CH), 3.16 (1H, dd, J = 15.0, 7.7 Hz, 3-CH), 2.00 (3H, s, $COCH_3$).
^{13}C NMR: δ 170.87, 169.96 (2xs, COOH, $O\underline{C}OCH_3$), 136.04, 127.28 (2xs, Ar), 123.84, 120.94, 118.43, 118.33, 111.39 (5xd, Ar), 108.90 (s, Ar), 72.70 (d, 3-$CH_2\underline{C}H$), 26.75 (t, 3-CH_2), 20.54 (q, CH_3).
HREIMS m/z calculated for $C_{13}H_{13}NO_4$:
247.0845 (M^+).
Found: 247.0848.

The above α-O-acetate (1.30 g of 90%, 4.4 mmol) and Et_3N (0.88 mL, 6.3 mmol) in DMF (10 mL) at 0°C was treated sequentially with DEPC (0.91 mL of 98%, 5.9 mmol) and benzylamine (0.69 mL, 6.3 mmol), and the mixture was stirred under nitrogen at 20°C for

18 hours. Workup and chromatography on silica gel, eluting with EtOAc/light petroleum (1:2 then 1:1) gave DL-α-acetoxy-*N*-benzyl-3-(3-indolyl)propanamide [II: R_1 = R_3 = H, R_2 = $CH_2CH(OAc)CONHCH_2Ph$] (0.29 g, 18%) as an oil.

^{1}H NMR ($CDCl_3$): δ 8.05 (1H, s, NH), 7.60 (1H, d, *J* = 7.9 Hz, ArH), 7.37 (1H, dt, *J* = 8.1, 0.9 Hz, ArH), 7.26-7.21 (3H, m, ArH), 7.20 (1H, ddd, *J* = 8.1, 7.0, 1.1 Hz, ArH), 7.12 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArH), 6.97 (1H, d, *J* = 2.4 Hz, H-2), 6.94 (2H, m, ArH), 6.07 (1H, t, *J* = 5.8 Hz, $N\underline{H}CH_2$), 5.47 (1H, t, *J* = 5.4 Hz, 3-$CH_2C\underline{H}$), 4.38 (1H, dd, *J* = 14.9, 6.1 Hz, NHC$\underline{H}$), 4.29 (1H, dd, *J* = 14.9, 5.5 Hz, NHC$\underline{H}$), 3.41 (2H, d, *J* = 5.5 Hz, 3-CH_2), 2.06 (3H, s, $COCH_3$).

^{13}C NMR: δ 169.63, 169.33 (2xs, CONH, O$\underline{C}$OCH$_3$), 137.56, 136.05 (2xs, Ar), 128.55 (d, 2C, Ar), 127.75 (s, Ar), 127.60 (d, 2C, Ar), 127.40, 123.43, 122.08, 119.61, 118.92, 111.13 (6xd, Ar), 109.83 (s, Ar), 74.56 (d, 3-$CH_2\underline{C}$H), 43.12 (t, $NHCH_2$), 27.42 (t, 3-CH_2), 21.09 (q, CH_3).

HREIMS *m/z* calculated for $C_{20}H_{20}N_2O_3$:

336.1474 (M^+).

Found: 336.1471.

Unreacted α-acetoxy-3-(3-indolyl)propanoic acid (0.68 g, 52%) was also recovered.

Alternative Preparation of Above Acetoxypropanamide

A solution of $SnCl_4$ (5.4 mL, 46 mmol) in CCl_4 (50 mL) was added dropwise to a stirred solution of indole (5.4 g, 46 mmol) and *N*-benzyl-2,3-epoxypropanamide (Dolzani L, Tamaro M, Monti-Bragadin C, Cavicchionz G, Vecchiati G, D'Angeli F, Mutation Res. 1986;172:37) (14 g of 85%, 67 mmol) in CCl_4 (100 mL) at -5°C (method of

Entzeroth M, Kunczik T, Jaenicke L, Liebig's Ann. Chim. 1983:226). The mixture was stirred at 20°C for 16 hours, then diluted with $CHCl_3$ (100 mL) and 10% $NaHCO_3$ (250 mL) and stirred vigorously for 4 hours. The aqueous portion was separated and extracted with CH_2Cl_2 (2 x 100 mL), and the combined organic extracts were washed with water, dried, and the solvents removed. The resulting oil was chromatographed on silica gel, eluting with CH_2Cl_2/light petroleum (1:1) to yield unreacted indole (1.27 g, 24%). Elution with CH_2Cl_2 gave mixtures, then CH_2Cl_2/EtOAc (4:1) gave a crude product. This was crystallized successively from CH_2Cl_2/light petroleum, then CH_2Cl_2/benzene/light petroleum to give DL-*N*-benzyl-α-hydroxy-3-(3-indolyl)-propanamide [II: $R_1 = R_3 = H$, $R_2 = CH_2CH(OH)CONHCH_2Ph$] (0.70 g, 5%); mp 127-128.5°C.

^1H NMR ($(CD_3)_2SO$): δ 10.79 (1H, s, NH), 8.20 (1H, t, J = 6.2 Hz, $N\underline{H}CH_2$), 7.56 (1H, d, J = 7.8 Hz, ArH), 7.34 (1H, d, J = 8.1 Hz, ArH), 7.24 (2H, m, ArH), 7.19 (1H, m, ArH), 7.12 (1H, d, J = 2.3 Hz, H-2), 7.10 (1H, m, ArH), 7.05 (1H, ddd, J = 8.0, 7.0, 1.0 Hz, ArH), 6.96 (1H, ddd, J = 7.9, 7.0, 0.9 Hz, ArH), 5.54 (1H, d, J = 5.7 Hz, OH), 4.26 (2H, d, J = 6.2 Hz, $NHC\underline{H}_2$), 4.19 (1H, ddd, J = 7.5, 5.7, 4.3 Hz, 3-$CH_2C\underline{H}$), 3.14 (1H, dd, J = 14.5, 4.1 Hz, 3-CH), 2.91 (1H, dd, J = 14.5, 7.6 Hz, 3-CH).

^{13}C NMR: δ 173.59 (s, CONH), 139.40, 135.93 (2xs, Ar), 128.00 (d, 2C, Ar), 127.60 (s, Ar), 126.95 (d, 2C, Ar), 126.42, 123.58, 120.56, 118.60, 117.97, 111.05 (6xd, Ar), 110.53 (s, Ar), 71.86 (d, 3-$CH_2\underline{C}H$), 41.60 (t, $NHCH_2$), 30.33 (t, 3-CH_2).

Analysis calculated for $C_{18}H_{18}N_2O_2 \cdot 0.25H_2O$ requires:
C, 72.4; H, 6.2; N, 9.4%.
Found: C, 72.4; H, 6.0; N, 9.3%.

This α-hydroxypropanamide (0.62 g, 2.1 mmol) was stirred with pyridine (1.5 mL, 18.5 mmol) and Ac_2O (1.7 mL, 18.0 mmol) at 20°C for 17 hours. The mixture was partitioned between water and CH_2Cl_2, and worked up to give a quantitative yield of DL-α-acetoxy-*N*-benzyl-3-(3-indolyl)propanamide [II: $R_1 = R_3 = H$, $R_2 = CH_2CH(OAc)CONHCH_2Ph$].

This compound (1.07 g) was treated with S_2Cl_2 as above, and the resulting product mixture chromatographed on silica gel, eluting with CH_2Cl_2/EtOAc (19:1), to give firstly 2,2'-thiobis-[α-acetoxy-*N*-benzyl-3-(3-indolyl)propanamide] [VI: $n = 1$, $R_1 = R_3 = H$, $R_2 = CH_2CH(OAc)CONHCH_2Ph$] (0.19 g, 17%) as a mixture of diastereoisomers; mp (MeOH/dilute HCl) 105-109°C.

^1H NMR ($CDCl_3$): δ 10.09, 10.06 (2x1H, 2xs, NH), 7.61, 7.60 (2x1H, 2xd, *J* = 7.9 Hz, ArH), 7.24 (2x1H, d, *J* = 8.2 Hz, ArH), 7.14-7.00 (2x5H, m, ArH), 6.78, 6.70 (2x2H, 2xm, ArH), 6.27, 6.26 (2x1H, 2xt, *J* = 5.8 Hz, $N\underline{H}CH_2$), 5.72 (1H, dd, *J* = 7.0, 6.0 Hz, 3-$CH_2C\underline{H}$), 5.69 (1H, t, *J* = 6.1 Hz, 3-$CH_2C\underline{H}$), 4.30, 4.27 (2x1H, 2xdd, *J* = 15.0, 5.8 Hz, NHC$\underline{H}$), 4.23, 4.21 (2x1H, 2xdd, *J* = 15.0, 5.4 Hz, NHC$\underline{H}$), 3.67 (1H, dd, *J* = 14.5, 7.0 Hz, 3-CH), 3.65 (1H, dd, *J* = 14.7, 5.8 Hz, 3-CH), 3.60 (1H, dd, *J* = 14.7, 6.3 Hz, 3-CH), 3.53 (1H, dd, *J* = 14.5, 6.0 Hz, 3-CH) 2.12, 2.11 (2x3H, 2xs, $COCH_3$).

^{13}C NMR ($CDCl_3$): δ 169.87, 169.73 (2xs, 2x2C, $COCH_3$, CONH), 137.09, 137.03, 136.70, 136.65 (4xs, Ar), 128.60, 128.56 (2xd, 2x2C, Ar), 127.48, 127.44 (2xd, Ar), 127.43, 127.39 (2xs, Ar), 127.31, 127.28 (2xd, 2x2C, Ar), 125.47, 125.40 (2xs, Ar), 122.95, 122.93 (2xd, Ar), 119.64 (d, 2C, Ar), 119.07, 118.88 (2xd, Ar), 113.92, 113.70 (2xs, Ar), 111.32 (d, 2C, Ar),

73.99, 73.77 (2xd, 3-CH_2CH), 43.31 (t, 2C, $NHCH_2$), 28.00 (t, 2C, 3-CH_2), 21.19, 21.13 (2xq, CH_3).
Analysis calculated for $C_{40}H_{38}N_4O_2S$) requires:
C, 68.4; H, 5.4; N, 8.0; S, 4.6%.
Found: C, 68.2; H, 5.6; N, 8.0; S, 4.8%.

Elution with CH_2Cl_2/EtOAc (9:1) gave 2,2'-dithiobis[α-acetoxy-*N*-benzyl-3-(3-indolyl)-propanamide] (**63**) [VI: n = 2, R_1 = R_3 = H, R_2 = $CH_2CH(OAc)CONHCH_2Ph$] (0.76 g, 65%) as a yellow oil (mixture of diastereoisomers). A subsample crystallized from CH_2Cl_2/dilute HCl as a single pair of diastereoisomers; mp 120-124°C (dec).
^{1}H NMR ($CDCl_3$): δ 8.64 (1H, s, NH), 7.60 (1H, d, *J* = 7.9 Hz, ArH), 7.27-7.15 (4H, m, ArH), 7.12, 7.11 (2x1H, 2xt, *J* = 8.1 Hz, ArH), 6.91 (2H, m, ArH), 6.12 (1H, t, *J* = 5.6 Hz, N<u>H</u>CH_2), 5.41 (1H, t, *J* = 6.2 Hz, 3-CH_2<u>CH</u>), 4.30, 4.24 (2x1H, 2xdd, *J* = 14.8, 5.71 Hz, NH<u>CH_2</u>), 3.31 (1H, dd, *J* = 14.5, 5.8 Hz, 3-CH), 3.17 (1H, dd, *J* = 14.5, 6.6 Hz, 3-CH), 1.99 (3H, s, $COCH_3$).
^{13}C NMR ($CDCl_3$): δ 169.65, 168.96 (2xs, CONH, <u>C</u>OCH_3), 137.50, 137.05 (2xs, Ar), 128.63 (d, 2C, Ar), 127.81 (s, Ar), 127.68 (d, 2C, Ar), 127.49 (d, Ar), 126.85 (s, Ar), 124.30, 120.30, 120.03 (3xd, Ar), 117.87 (s, Ar), 111.33 (d, Ar), 74.06 (d, 3-CH_2<u>C</u>H), 43.30 (t, $NHCH_2$), 27.45 (t, 3-CH_2), 21.18 (q, CH_3).
Analysis calculated for $C_{40}H_{38}N_4O_2S_2$ requires:
C, 65.4; H, 5.2; N, 7.6; S, 8.7%.
Found: C, 65.2; H, 5.2, N, 7.8; S, 8.8%.

<u>Compound 64 of Table 1</u>

Hydrolysis of **63** with excess $KHCO_3$ in aqueous MeOH at 20°C for 2 hours gave 2,2'-dithiobis[α-hydroxy-N-(phenylmethyl)-1H-indole-3-propanamide] (**64**) [II: R_1 = R_3 = H, R_2 = $Ch_2CH(OH)COOH$] as an oil

(mixture of diastereomers) in essentially quantitative yield. Crystallization from CH_2Cl_2/light petroleum gave a single pair of diastereomers (66% yield); mp 120-125°C.

^1H NMR ($CDCl_3$): δ 7.61 (1H, d, J = 8.0 Hz, ArH), 7.33-7.17 (5H, m, ArH), 7.12 (2H, dd, J = 7.8, 1.5 Hz, ArH), 7.09 (1H, ddd, J = 8.1, 5.4, 2.7 Hz, ArH), 6.80 (1H, t, J = 5.8 Hz, $NHCH_2$), 4.33, 4.27 (2x1H, 2xdd, J = 14.8, 5.9 Hz, $NHCH_2$), 3.78 (1H, ddd, J = 9.5, 5.4, 3.4 Hz, 3-CH_2CH), 3.30 (1H, d, J = 5.4 Hz, OH), 3.24 (1H, dd, J = 14.4, 3.4 Hz, 3-CH), 2.88 (1H, dd, J = 14.3, 9.5 Hz, 3-CH).

Analysis calculated for $C_{36}H_{34}N_4O_4S_2$ requires:
C, 66.1; H, 5.3; N, 8.6; S, 9.6%.
Found: C, 66.5; H, 5.2; N, 8.6; S, 9.8%

EXAMPLE C

Preparation of Compounds 5 and 33 of Table 1 by the Method Outlined in Scheme 3

1-Methyl-2-indolinone [VII: R_1 = H, R_3 = Me] was condensed with diethyl oxalate in NaOEt/EtOH, to give ethyl 1-methyl isatylidenehydroxyacetate [VIII: R_1 = H, R_3 = Me, R = COOEt] (82% yield); mp 62-64°C (according to the method of Porter JC, Robinson R, Wyler M, J. Chem. Soc. 1941:620, who report mp 81°C). The above acetate [VIII: R_1 = H, R_3 = Me, R = COOEt] (2.30 g) was hydrogenated in glacial AcOH (150 mL) containing concentrated H_2SO_4 (1 mL) and 5% Pd/C catalyst (5 g) for 1 day. The reaction mixture was filtered onto NaOAc (4 g) and the solvent removed under reduced pressure. The residue was partitioned between CH_2Cl_2 and water, then the aqueous phase re-extracted with CH_2Cl_2. The CH_2Cl_2 extracts were combined, washed with water, the solvent removed, and

the residue was chromatographed on silica gel. Elution with CH_2Cl_2 gave ethyl 2-(1-methyl-2-oxo-3-indolinyl)-acetate [III: R_1 = H, R_2 = CH_2COOEt, R_3 = Me] as an oil (1.23 g, 57%).

^1H NMR ($CDCl_3$): δ 7.29 (1H, t, J = 7.7 Hz, ArH), 7.26 (1H, d, J = 7.5 Hz, ArH), 7.03 (1H, t, J = 7.5 Hz, ArH), 6.84 (1H, d, J = 7.7 Hz, ArH), 4.15, 4.11 (2x1H, 2xdq, J = 10.8, 7.1 Hz, $COOCH_2$), 3.79 (1H, dd, J = 8.0, 4.4 Hz, H-3), 3.23 (3H, s, NCH_3), 3.07 (1H, dd, J = 16.8, 4.4 Hz, CH_2CO), 2.78 (1H, dd, J = 16.8, 8.1 Hz, CH_2CO), 1.20 (3H, t, J = 7.1 Hz, $OCH_2\underline{C}H_3$).

^{13}C NMR ($CDCl_3$): δ 176.72 (s, $\underline{C}ONCH_3$), 171.02 ($\underline{C}OOCH_2$) 144.35 (s, ArH), 128.27 (d, ArH), 128.18 (s, ArH), 123.80, 122.45, 108.01 (3xd, ArH), 60.85 (t, OCH_2), 41.83 (d, C-3), 34.94 (t, $\underline{C}H_2CO$), 26.28 (q, NCH_3), 14.05 (q, $OCH_2\underline{C}H_3$).

The above oxoacetate [III: R_1 = H, R_2 = CH_2COOEt, R_3 = Me] was treated with P_2S_5 as described in Example A, then chromatographed on silica gel, with CH_2Cl_2/light petroleum (3:2) eluting ethyl 2-(1-methyl-2-thioxo-3-indolinyl)acetate [IV: R_1 = H, R_2 = CH_2COOEt, R_3 = Me] (5) (90% yield); mp (benzene/light petroleum) 47-48°C.

^1H NMR ($CDCl_3$): δ 7.35 (2H, m, ArH), 7.16 (1H, td, J = 7.5, 0.8 Hz, ArH), 7.01 (1H, dd, J = 7.7, 1.0 Hz, ArH), 4.15 (2H, q, J = 7.1 Hz, $COOCH_2$), 4.14 (1H, m, H-3), 3.65 (3H, s, NCH_3), 3.39 (1H, dd, J = 17.0, 4.1 Hz, CH_2CO), 2.83 (1H, dd, J = 17.0, 8.6 Hz, CH_2CO), 1.22 (3H, t, J = 7.1 Hz, $OCH_2\underline{C}H_3$).

^{13}C NMR ($CDCl_3$): δ 204.35 (s, $\underline{C}SNCH_3$), 171.11 (s, $\underline{C}OOCH_2$), 145.73, 133.01 (2xs, ArH), 128.39, 124.34, 123.94, 109.46 (4xd, ArH), 60.85 (t, OCH_2), 53.44 (d, C-3), 38.66 (t, $\underline{C}H_2CO$), 31.52 (q, NCH_3), 14.13 (q, $OCH_2\underline{C}H_3$).

Analysis calculated for $C_{13}H_{15}NO_2S$ requires:
C, 62.7; H, 6.0; N, 5.6; S, 12.9%.
Found: C, 62.5; H, 6.2; N, 5.6; S, 12.8%.

A solution of crude **5** in EtOH was exposed to air for 2 weeks, during which time bis[ethyl 1-methylindolyl-3-acetate-(2)]disulfide [V: R_1 = H, R_2 = CH_2COOEt, R_3 = Me] (**33**) slowly separated as yellow needles (0.18 g, 26%); mp 117-119°C.
^1H NMR ($CDCl_3$): δ 7.53 (1H, dt, J = 8.0, 0.8 Hz, ArH), 7.30 (1H, ddd, J = 8.3, 6.3, 1.1 Hz, ArH), 7.27 (1H, ddd, J = 8.1, 1.6, 0.7 Hz, ArH), 7.12 (1H, ddd, J = 8.0, 6.2, 1.8 Hz, ArH), 3.96 (2H, q, J = 7.1 Hz, $COOCH_2$), 3.54 (3H, s, NCH_3), 3.38 (2H, s, CH_2CO), 1.14 (3H, t, J = 7.1 Hz, OCH_2CH_3).
^{13}C NMR ($CDCl_3$): δ 171.06 (s, $\underline{C}OOCH_2$), 138.45, 128.42, 126.47 (3xs, ArH), 124.33, 120.20, 120.07 (3xd, ArH), 117.59 (s, ArH), 109.93 (d, ArH), 60.70 (t, OCH_2), 30.99 (t, $\underline{C}H_2CO$), 29.97 (q, NCH_3), 14.13 (q, $OCH_2\underline{C}H_3$).
Analysis calculated for $C_{26}H_{28}N_2O_4S_2$ requires:
C, 62.9; H, 5.7; N, 5.7; S, 12.9%.
Found: C, 62.7; H, 5.6; N, 5.6; S, 13.0%.

Compounds 10 and 38 of Table 1

Similar reactions on 2-indolinone [VII: R_1 = R_3 = H], using diethyl malonate, gave ethyl 3-(2-oxo-3-indolinyl)propanoate [III: R_1 = R_3 = H, R_2 = $(CH_2)_2COOE$] (Julian PL, Printy HC, J. Am. Chem. Soc. 1953;75:5301). Reaction of this with P_2S_5 as described in Example A, followed by chromatography on silica gel, elution with CH_2Cl_2, and crystallization from benzene/light petroleum over 2 days, gave bis[ethyl indolyl-3-propanoate-(2)]disulfide [V: R_1 = R_3 = H, R_2 = $(CH_2)_2COOEt$] (**38**) (18% yield); mp 137-139°C.

^{1}H NMR ($CDCl_3$): δ 8.25 (1H, s, NH), 7.55 (1H, d, J = 8.0 Hz, ArH), 7.22 (2H, m, ArH), 7.11 (1H, ddd, J = 8.0, 5.0, 3.0 Hz, ArH), 4.02 (2H, q, J = 7.1 Hz, $COOCH_2$), 2.98, 2.46 (2x2H, 2xt, J = 7.9 Hz, CH_2CH_2CO), 1.16 (3H, t, J = 7.1 Hz, OCH_2CH_3).

^{13}C NMR ($CDCl_3$): δ 173.03 (s, $COOCH_2$), 137.26, 127.22, 125.83 (3xs, ArH), 124.26 (d, ArH), 122.81 (s, ArH), 120.03, 119.63, 111.19 (3xd, ArH), 60.41 (t, $COOCH_2$), 35.20 (t, CH_2CO), 20.26 (t, 3-CH_2), 14.14 (q, OCH_2CH_3).

Analysis calculated for $C_{26}H_{28}N_2O_4S_2$ requires:

C, 62.9; H, 5.7; N, 5.6; S, 12.9%.

Found: C, 63.3; H, 5.9; N, 5.7; S, 13.0%.

Treatment of the mother liquors with $NaBH_4$ gave ethyl 3-(2-thioxo-3-indolinyl)propanoate [IV: $R_1 = R_3 = H$, $R_2 = (CH_2)_2COOEt$] (**10**) (56% yield) as an oil.

^{1}H NMR ($CDCl_3$): δ 10.40 (1H, s, NH), 7.31 (1H, d, J = 7.4 Hz, ArH), 7.27 (1H, td, J = 7.8, 0.7 Hz, ArH), 7.14 (1H, td, J = 7.5, 0.7 Hz, ArH), 7.01 (1H, d, J = 7.8 Hz, ArH), 4.07, 4.03 (2x1H, 2xdq, J = 10.8, 7.1 Hz, $COOCH_2$), 3.91 (1H, t, J = 5.4 Hz, H-3), 2.52 (2H, m, CH_2CH_2CO), 2.41 (1H, ddd, J = 15.8, 9.9, 5.9 Hz, CH_2CO), 2.10 (1H, ddd, J = 15.8, 9.1, 6.7 Hz, CH_2CO), 1.20 (3H, t, J = 7.1 Hz, OCH_2CH_3).

^{13}C NMR ($CDCl_3$): δ 207.31 (s, CSNH), 172.96 (s, $COOCH_2$), 143.31, 133.15 (2xs, ArH), 128.40, 124.34, 124.07, 110.04 (4xd, ArH), 60.55 (t, OCH_2), 56.44 (d, C-3), 29.56, 28.16 (2xt, $(CH_2)_2CO$), 14.15 (q, OCH_2CH_3).

Analysis calculated for $C_{13}H_{15}NO_2S$ requires:

C, 62.6; H, 6.1; N, 5.6; S, 12.9%.

Found: C, 62.3; H, 5.9; N, 5.6; S, 12.6%.

Compounds 12 of Table 1

Similar treatment of 1-methyl-2-indolinone, using diethyl malonate, and subsequent thiation, gave ethyl 3-(1-methyl-2-thioxo-3-indolinyl)propanoate [IV: R_1 = H, R_2 = $(CH_2)_2COOEt$, R_3 = Me] (**12**); mp (benzene/light petroleum) 61-63°C.

^1H NMR ($CDCl_3$): δ 7.35 (2H, m, ArH), 7.20 (1H, t, J = 7.5 Hz, ArH), 7.00 (1H, d, J = 7.8 Hz, ArH), 4.05, 4.02 (2x1H, 2xdq, J = 10.8, 7.1 Hz, $COOCH_2$), 3.92 (1H, t, J = 5.4 Hz, H-3), 3.63 (3H, s, NCH_3), 2.53 (2H, td, J = 8.0, 5.4 Hz, $C\underline{H}_2CH_2CO$), 2.32, 2.01 (2x1H, 2xtd, J = 16.0, 8.0 Hz, $CH_2C\underline{H}_2CO$), 1.19 (3H, t, J = 7.1 Hz, $CH_2C\underline{H}_3$).

^{13}C NMR ($CDCl_3$): δ 204.85 (s, $\underline{C}SNCH_3$), 172.87 (s, $\underline{C}OOCH_2$), 145.89, 132.44 (2xs, ArH), 128.37, 124.30, 124.00, 109.49 (4xd, ArH), 60.43 (t, OCH_2), 56.29 (d, C3), 31.35 (q, NCH_3), 29.53, 28.46 (2xt, CH_3CH_2CO), 14.15 (q, $OCH_2\underline{C}H_3$).

Analysis calculated for $C_{14}H_{17}NO_2S$ requires:
C, 63.9; H, 6.5; N, 5.3; S, 12.2%.
Found: C, 64.1; H, 6.7; N, 5.4; S, 12.0%.

Compounds 41 and 42 of Table 1

Similar treatment of 5-methyl-2-indolinone [VII: R_1 = 5-Me, R_3 = H] gave bis[ethyl 5-methylindolyl-3-propanoate-(2)]disulfide [V: R_1 = 5-Me, R_2 = $(CH_2)_2COOEt$, R_3 = H] (**42**) as a yellow solid; mp (benzene/petroleum ether) 138.5-139°C.

^1H NMR ($CDCl_3$): 8.10 (1H, s, NH), 7.32 (1H, d, J = 0.6 Hz, H-4), 7.15 (1H, d, J = 8.3 Hz, H-7), 7.06 (1H, dd, J = 8.3, 1.4 Hz, H-6), 4.03 (2H, q, J = 7.2 Hz, $C\underline{H}_2CH_3$), 3.02-2.85 (2H, m, $CH_2CH_2CO_2$), 2.51-2.36 (2H, m, $CH_2CH_2CO_2$), 2.43 (3H, s, $ArCH_3$), 1.18 (3H, t, J = 7.2 Hz, $CH_2C\underline{H}_3$).

^{13}C NMR ($CDCl_3$): δ 173.1 (CO_2Et), 135.6, 129.3, 127.4, 125.9, 122.3 (C-2,3,5,8,9), 126.0, 119.1, 110.9 (C-4,6,7), 60.4 ($O\underline{C}H_2CH_3$), 35.2 ($\underline{C}H_2CH_2CO2$), 21.5 ($ArCH_3$), 20.3 ($CH_2\underline{C}H_2CO2$), 14.1 ($OCH_2\underline{C}H_3$).

Analysis calculated for $C_{28}H_{32}N_2O_4S_2 \cdot 0.5C_6H_6$ requires: C, 66.1; H, 6.3; N, 5.0; S, 11.4%.

Found: C, 66.2; H, 6.4; N, 5.0; S, 11.7%.

Ester hydrolysis of **42** as above gave bis[5-methylindolyl-3-propanoic acid-(2)]disulfide [V: R_1 = 5-Me, R_2 = $(CH)_2CO_2H$, R_3 = H] (**41**) as orange-brown prisms; mp (CH_2Cl_2/petroleum ether) 91.5-95°C.

^1H NMR ($CDCl_3$): δ 7.98 (1H, s, NH), 7.33 (1H, s, H-4), 7.14 (1H, d, J = 8.4 Hz, H-7), 7.07 (1H, dd, J = 8.4, 1.3 Hz, H-6), 2.98 (2H, t, J = 7.5 Hz, $CH_2CH_2CO_2$), 2.56 (2H, t, J = 7.5 Hz, $CH_2CH_2CO_2$), 2.43 (3H, s, $ArCH_3$).

HREIMS m/z calculated for $C_{24}H_{24}N_2O_4S_2$ requires: 235.06670.

Found: m/z 235.06639.

<u>Compounds 43 and 44 of Table 1</u>

Similar treatment of 6-methyl-2-indolinone [VII: R_1 = 6-Me, R_3 = H] gave bis[ethyl 6-methylindolyl-3-propanoate-(2)]disulfide [V: R_1 = 6-Me, R_2 = $(CH_2)_2COOEt$, R_3 = H] (**44**) as a yellow solid; mp 122-123.5°C.

^1H NMR ($CDCl_3$): δ 8.06 (1H, s, NH), 7.43 (1H, d, J = 8.2 Hz, H-4), 7.03-7.00 (1H, m, H-7), 6.97-6.92 (1H, m, H-5), 4.02 (2H, q, J = 7.2 Hz, $C\underline{H}_2CH_3$), 2.98-2.91 (2H, m, CH_2CH_2CO), 2.48-2.42 (2H, m, CH_2CH_2CO), 2.44 (3H, s, $ArCH_3$), 1.17 (3H, t, J = 7.2 Hz, $CH_2C\underline{H}_3$).

^{13}C NMR ($CDCl_3$): δ 173.0 (CO_2Et), 137.7, 134.3, 125.2, 125.0, 122.9 (C-2,3,6,8,9), 121.9, 119.3 (C-4,5,7),

60.3 ($O\underline{C}H_2CH_3$), 35.2 ($\underline{C}H_2CH_2CO_2$), 21.8 ($ArCH_3$), 20.3 ($CH_2\underline{C}H_2CO_2$), 14.1 ($OCH_2\underline{C}H_3$).

Analysis calculated for $C_{28}H_{32}N_2O_4S_2$ requires:

C, 64.1; H, 6.2; N, 5.3; S, 12.2%.

Found: C, 64.1; H, 6.2; N, 5.4; S, 12.0%.

Ester hydrolysis of the above as above gave bis[methylindolyl-3-propanoate-(2)]disulfide [V: R_1 = 6-Me, R_2 = $(CH_2)_2COOEt$, R_3 = H] (**43**) as yellow microcrystals; mp (CH_2Cl_2/petroleum ether) 126-128°C.

^1H NMR ($(CD3)_2CO$): δ 10.34 (1H, br s, NH), 7.49 (1H, d, J = 8.2 Hz, H-4), 7.19 (H, s, H-7), 6.19 (1H, dd, J = 8.2, 1.2 Hz, H-5), 2.97-2.90 (2H, m, $CHCH_2CO_2$), 2.49-2.43 (2H, m, $CH_2CH_2CO_2$), 2.42 (3H, s, $ArCH_3$).

Analysis calculated for $C_{24}H_{24}N_2O_4S_2 \cdot H_2O$ requires:

C, 60.4; H, 5.9; N, 5.9%.

Found: C, 60.2; H, 5.3; N, 5.9%.

<u>Compounds **45** and **46** of Table 1</u>

Similar treatment of 7-methyl-2-indolinone [VII: R_1 = 7-Me, R_3 = H] gave bis[ethyl 7-methylindolyl-3-propanoate-(2)]disulfide [V: R_1 = 7-Me, R_2 = $(CH_2)_2COOEt$, R_3 = H] (**46**) as a yellow solid; mp (benzene/petroleum ether) 120-122.5°C.

^1H NMR ($CDCl_3$): δ 8.23 (1H, s, NH), 7.38 (1H, d, J = 7.4 Hz, ArH), 7.00 (1H, t, J = 7.3 Hz, H-5), 6.94 (1H, d, J = 6.3 Hz, ArH), 4.02 (2H, q, J = 7.2 Hz, $C\underline{H}_2CH_3$), 3.16 (2H, t, J = 7.5 Hz, $CH_2CH_2CO_2$), 2.71 (2H, t, J = 7.5 Hz, $CH_2CH_2CO_2$), 1.96 (3H, s, $ArCH_3$), 1.23 (3H, t, J = 7.2 Hz, $CH_2C\underline{H}_3$).

^{13}C NMR ($CDCl_3$): δ 173.6 (CO_2Et), 136.9, 127.0, 124.8, 122.9, 121.0 (C-2,3,7,8,9), 124.3, 120.0, 117.0 (C-4,5,6), 60.6 ($O\underline{C}H_2CH_3$), 35.3 ($\underline{C}H_2CH_2CO_2$), 20.9 ($CH_2\underline{C}H_2CO_2$), 16.0 ($ArCH_3$), 14.1 ($OCH_2\underline{C}H_3$).

Analysis calculated for $C_{28}H_{32}N_2O_4S_2$ requires:
C, 64.1; H, 6.2; N, 5.3; S, 12.2%.
Found: C, 64.2; H, 6.4; N, 5.4; S, 12.0%.

Ester hydrolysis of **46** as above gave bis[7-methylindolyl-3-propanoic acid-(2)]disulfide [V: R_1 = 7-Me, R_2 = $(CH_2)_2CO2H$, R_3 = H] (**45**) as green needles; mp (AcOH/petroleum ether) 172.5-175°C. ^1H NMR ($(CD3)_2CO$): δ 10.37 (1H, br s, NH), 7.45 (1H, d, J = 7.0 Hz, ArH), 7.03-6.95 (2H, m, ArH), 3.01-2.94 (2H, m, $CH_2CH_2CO_2$), 2.50-2.42 (2H, m, $CH_2CH_2CO_2$), 2.49 (3H, s, $ArCH_3$).
Analysis calculated for $C_{24}H_{24}N_2O_4S_2$ requires:
C, 61.5; H, 5.2; N, 6.0%.
Found: C, 61.3; H, 5.1; N, 6.0%.

EXAMPLE D

Preparation of Compounds **21-23** and **70** of Table 1 by the Method Outlined in Scheme 4

Powdered Na_2CO_3 (0.70 g, 6.61 mmol) was added to a suspension of P_2S_5 (2.93 g, 6.61 mmol) in THF (40 mL) and the mixture was stirred vigorously at 20°C until homogeneous, and gas evolution had ceased (15 minutes). A solution of 1-methyl-2-indolinone [VII: R_1 = R_3 = Me] (0.80 g, 5.50 mmol) in THF (10 mL) was added and stirring was continued for 18 hours. After pouring into brine, the mixture was extracted into EtOAc, worked up, and chromatographed on silica. Elution with EtOAc/petroleum ether (1:4) gave 1-methyl-2-indolinethione [IX: R_1 = R_3 = Me] (0.71 g, 87%); mp 108-109°C (Hino T, Tsuneoka K, Nakagawa M, Akaboshi S, Chem. Pharm. Bull. 1969;17:550 record 109-111°C).

A solution of the above 1-methyl-2-indolinethione (4.1 g) in THF (150 mL) was treated dropwise over

15 minutes with an ice-cooled suspension of NaH (57%, 1.4 g) in THF (100 mL). The mixture was stirred for 30 minutes, then a solution of phenyl isocyanate (3.5 g) in THF (50 mL) was added, and stirring continued for 3 hours at 20°C. The solvent was removed under vacuum, then the residue decomposed with ice-HCl, and extracted in CH_2Cl_2. Removal of the solvent gave an oil (6.0 g), which crystallized from ether. Two recrystallizations from THF-ether gave N-phenyl (1-methyl-2-thioxo-3-indolinyl)carboxamide [IV: R_1 = H, R_2 = CONHPh, R_3 = Me] (**21**) (2.8 g, 39%) as a pale yellow solid; mp 149-151°C.

^1H NMR ($CDCl_3$): δ 10.36 (1H, s, NH), 7.87 (1H, d, J = 7.4 Hz, ArH), 7.60 (2H, d, J = 7.9 Hz, ArH), 7.41 (2H, t, J = 7.5 Hz, ArH), 7.31 (2H, m, ArH), 7.11 (1H, t, J = 7.3 Hz, ArH), 7.03 (1H, d, J = 7.8 Hz, ArH), 3.73 (3H, s, NCH_3).

Analysis calculated for $C_{16}H_{14}N_2OS$ requires:
C, 68.1; H, 5.1; N, 9.9; S, 11.4%.
Found: C, 67.8; H, 5.1; N, 9.8; S, 11.4%.

A solution of **21** (200 mg) in CH_2Cl_2/MeOH (2:1) (30 mL) was stirred at 20°C for 5 days, then the solvents were removed under reduced pressure. Chromatography on silica gel, eluting with CH_2Cl_2 then $CHCl_3$/EtOH (99:1), gave bis[N-phenyl 1-methylindolyl-3-carboxamide-(2)] disulfide [V: R_1 = H, R_2 = CONHPh, R_3 = Me] (**70**) (0.19 g, 95%); mp (benzene) 187-188°C.

^1H NMR ($CDCl_3$): δ 8.21 (1H, s, NH), 8.01 (1H, d, J = 8.1 Hz, ArH), 7.19 (1H, ddd, J = 8.1, 7.1, 0.9 Hz, ArH), 7.13 (4H, d, J = 4.3 Hz, Ph), 7.09 (1H, ddd, J = 8.1, 7.1, 0.9 Hz, ArH), 7.05 (1H, d, J = 8.1 Hz, ArH), 6.98 (1H, quin, J = 4.3 Hz, Ph), 3.77 (3H, s, NCH_3).

^{13}C NMR ($CDCl_3$): δ 161.57 (CO), 138.55, 137.95 (2xs), 128.64 (d), 127.41, 126.07 (2xs), 125.55, 122.28, 122.00 (4xd), 119.76 (s), 119.27, 110.14 (2xd), 30.33 (NCH_3).

Analysis calculated for $C_{32}H_{26}N_4O_2S_2$ requires:
C, 68.3; H, 4.6; N, 10.0; S, 11.4%.
Found: C, 68.9; H, 4.9; N, 9.6; S, 11.1%.

A solution of **21** (200 mg) in Me_2CO (20 mL) was treated with K_2CO_3 (0.12 g) and methyl iodide (0.14 g) and the mixture stirred at 20°C for 1 hour. CH_2Cl_2 (100 mL) was added, then the solution filtered and the solvents removed, to yield a brown oil (0.26 g). Chromatography on silica gel, eluting with CH_2Cl_2, gave N-phenyl (1-methyl-2-methylthio-3-indolyl)carboxamide as an oil [X: R_1 = H, R_2 = CONHPh, R_3 = Me, R4 = SMe] (**22**) (200 mg, 95%), which crystallized from MeOH/CH_2Cl_2 as a white solid; mp 116-118°C.

^1H NMR ($CDCl_3$): δ 9.99 (1H, s, NH), 8.58 (1H, d, J = 8.0 Hz, ArH), 7.75 (2H, d, J = 7.6 Hz, ArH), 7.38 (4H, m, ArH), 7.29 (1H, quin, J = 4.3 Hz, ArH), 7.12 (1H, t, J = 7.4 Hz, ArH), 3.95 (3H, s, NCH_3), 2.47 (3H, s, SCH_3).

^{13}C NMR ($CDCl_3$): δ 162.59 (s, CONH), 138.80, 137.46, 131.43 (3xs, ArH), 129.03 (2xd, ArH), 127.35 (s, ArH), 124.14, 123.67, 123.02, 122.24 (4xd, ArH), 119.86 (2xd, ArH), 114.04 (s, ArH), 109.69 (d, ArH), 30.23 (q, NCH_3), 20.50 (q, SCH_3).

Analysis calculated for $C_{17}H_{16}N_2OS$ requires:
C, 68.9; H, 5.4; N, 9.5; S, 10.8%.
Found: C, 68.6; H, 5.5; N, 9.4; S, 10.8%.

Benzyl mercaptan (0.02 mL, 0.178 mmol) was added to a suspension of **70** (50 mg, 89 mmol) and BF3-etherate (1 drop) in CH_2Cl_2 (1 mL). After stirring at 20°C for 3 hours, the homogeneous mixture was poured into

saturated aqueous NaHCO3, diluted with CH_2Cl_2 and worked up, and the residue was chromatographed on silica gel. Elution with CH_2Cl_2/petroleum ether (1:1) gave foreruns, and elution with CH_2Cl_2 elute benzyl [N-phenyl 1-methylindolyl-3-carboxamide-(2)]disulfide [XI: R_1 = H, R_2 = CONHPh, R_3 = Me, R4 = S_2CH_2Ph] (**23**) (39 mg, 54%); mp ($CHCl_3$/petroleum ether) 146-148°C.

^1H NMR: δ 8.95 (1H, br s, CONH), 8.47 (1H, dd, J = 7.7, 1.3 Hz, ArH-4), 7.66 (2H, dd, J = 7.5, 1.2 Hz, Ph), 7.40-7.07 (11H, m, ArH-5,-6,-7 and Ph), 3.90 (3H, s, NMe).

^{13}C NMR: δ 162.31 (CONHPh), 138.31 (s), 138.04 (s), 135.13 (s), 130.00 (s), 129.15, 129.06, 128.69, 127.83, 126.83 (s), 124.79, 123.94, 122.80, 122.36, 119.90, 109.92, 42.51 (CH_2Ph), 30.73 (NCH_3).

Analysis calculated for $C_{23}H_{20}N_2S_2O$ requires:
C, 68.3; H, 5.0; N, 6.9; S, 15.9%.
Found: C, 68.4; H, 5.1; N, 6.9; S, 16.0%

Compound 71 of Table 1

Similarly was prepared, from 1-ethyl-2-indolinethione (Kendall JD, Ficken GE, British Patent 829,584, Chem. Abstr. 1960;54:12847h) and phenyl isocyanate, bis[N-phenyl 1-ethylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = H, R_2 = CONHPh, R_3 = Et] (**71**) (25% yield); mp 200-202°C.

^1H NMR ($CDCl_3$): δ 8.22 (1H, br, CONH), 7.98 (1H, d, J = 8.1 Hz, H-4), 7.18 (1H, t, J = 8.0 Hz, H-6), 7.11-7.04 (6H, m, H-5 and Ph), 6.95 (1H, dd, J = 8.0, 1.0 Hz, H-7), 4.32 (2H, q, J = 7.0 Hz, NCH_2CH_3), 1.36 (3H, t, J = 7.0 Hz, NCH_2CH_3).

^{13}C NMR: δ 161.73 (CONH), 137.91 (s), 137.44 (s), 128.55, 128.55, 128.35 (2s), 126.33 (s), 125.41,

123.47, 122.12, 122.07, 119.37, 110.19 (C-7), 38.86 ($N\underline{C}H_2CH_3$), 15.23 ($NCH_2\underline{C}H_3$).

Analysis calculated for $C_{34}H_{30}N_4S_2O_2$ requires:

C, 69.1; H, 5.1; N, 9.5; S, 10.8%.

Found: C, 68.9; H, 5.4; N, 9.5; S, 10.4%.

Compound 72 of Table 1

Similarly was prepared 4-chloro-1-methyl-2-indolinethione [IX: R_1 = 4-Cl, R_3 = Me] (92% yield); mp 147.5-149.5°C.

^1H NMR ($CDCl_3$): δ 7.29 (1H, t, J = 8.0 Hz, H-6), 7.13 (1H, d, J = 8.0 Hz, H-5), 6.86 (1H, d, J = 8.0 Hz, H-7), 4.09 (2H, s, H-3), 3.60 (3H, s, NCH_3).

^{13}C NMR: δ 200.75 (C-2), 147.65 (s), 130.04 (s), 129.52, 127.44 (s), 124.34, 107.81 (C-7), 48.42 (C-3), 31.55 (NCH_3).

Analysis calculated for C_9H_8ClNS requires:

C, 54.7; H, 4.1; N, 7.1; S, 16.2%.

Found: C, 54.5; H, 4.3; N, 7.1; S, 16.0%.

Reaction of this with phenyl isocyanate as above gave bis[N-phenyl 4-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 4-Cl, R_2 = CONHPh, R_3 = Me] (72) (21% yield); mp 225-228°C.

^1H NMR ($CDCl_3$): δ 8.38 (1H, br, NH), 7.49 (1H, dd, J = 7.9, 1.5 Hz, H-5), 7.12 (1H, t, J = 7.9 Hz, H-6), 7.08-7.05 (4H, m, CONH$\underline{Ph}$), 6.98 (1H, dd, J = 7.9, 1.5 Hz, H-7), 6.96 (1H, m, CONH$\underline{Ph}$), 3.77 (3H, S, N-CH_3).

Analysis calculated for $C_{32}H_{24}Cl_2N_4O_2S_2$ requires:

C, 60.8; H, 3.8; N, 8.9; Cl, 11.2%.

Found: C, 60.7; H, 4.1; N, 8.7; Cl, 11.8%.

Compound 73 of Table 1

Similarly was prepared, from 5-chloro-1-methyl-2-indolinethione [IX: R_1 = 5-Cl, R_3 = Me]; mp 163-165°C (Baudin J-B, Julia SA, Lorne R, Bull. Soc. Chim. Fr. 1987:181-188 records mp 153-155°C) and phenyl isocyanate, bis[N-phenyl 5-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 5-Cl, R_2 = CONHPh, R_3 = Me] (73) (27% yield); mp 214-216°C. ^{1}H NMR ($CDCl_3$): δ 8.14 (1H, br, CONH), 7.94 (1H, d, J = 1.8 Hz, H-4), 7.12 (4H, br, ArH), 7.07 (1H, d, J = 8.4 Hz, ArH), 7.01 (1H, m, ArH), 6.90 (1H, d, J = 8.9 Hz, ArH), 3.76 (3H, s, NCH_3). ^{13}C NMR: δ 161.06 (CONH), 137.72 (s), 136.81 (s), 128.73, 128.44 (s), 128.25 (s), 126.58 (s), 126.11, 123.76, 121.27, 119.71 (s), 118.80, 111.16 (C-7), 30.53 (NCH_3).

Analysis calculated for $C_{32}H_{24}Cl_2N_4O_2S_2$ requires:
C, 60.8; H, 3.8; N, 8.9; S, 10.2%.
Found: C, 60.6; H, 4.0; N, 8.9; S, 10.2%.

$NaBH_4$ (14 mg, 0.38 mmol) was added to a stirred suspension of the above compound (0.12 g, 0.19 mmol) in MeOH (5 mL). After 15 minutes, the solution was concentrated to dryness and the residue was partitioned between EtOAc and water. The organic solution was worked up to give a solid which was recrystallized from degassed $CHCl_3$/benzene at -5°C to give *N*-phenyl 5-chloro-1-methyl-2-thioxoindole-3-carboxamide (20) [IV: R_1 = 5-Cl, R_2 = CONHPh, R_3 = Me] as coarse needles (86% yield); mp 312-320°C (dec). ^{1}H NMR ($(CD_3)_2SO$): δ 12.84 (1H, s, SH), 8.09 (1H, d, *J* = 2.2 Hz, H-4), 7.70 (2H, d, *J* = 8.5 Hz, H-2',6'), 7.27 (2H, dd, J = 8.5, 8.2 Hz, H-3',5'), 7.07 (1H, d, *J* = 8.4 Hz, H-7), 6.92 (1H, t, *J* = 8.2 Hz, H-4'), 6.86 (1H, dd, *J* = 8.4, 2.2 Hz, H-6), 3.64 (3H, s, N-CH_3).

^{13}C NMR: δ 164.73 (CONH), 140.81 (s), 135.17 (s), 130.29 (s), 128.55 (d), 123.93 (s), 121.01 (d), 118.20 (d), 117.65 (d), 117.30 (d), 107.97 (d), 104.40 (s), 29.18 ($N-CH_3$).

Analysis calculated for $C_{16}H_{13}ClN_2OS$ requires:
M+ 318.0408, 316.0437.
Found: M+ (mass spectrum) 318.0414, 316.0431.

Compound 74 of Table 1

Similarly was prepared, from 7-chloro-1-methyl-2-indolinethione [IX: R_1 = 7-Cl, R_3 = Me]; mp 126-128°C (Inoue S, Uematsu T, Kato T, Ueda K, Pestic. Sci. 1985;16:589-598 records mp 125-127°C) and phenyl isocyanate, bis[N-phenyl-7-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 7-Cl, R_2 = CONHPh, R_3 = Me] (**74**) (27% yield); mp 232-234°C.

^1H NMR ($CDCl_3$): δ 8.15 (1H, br, CONH), 7.85 (1H, d, J = 8.0 Hz, H-4), 7.19-7.05 (5H, m, ArH), 7.00 (1H, t, J = 6.6 Hz, ArH), 6.90 (1H, t, J = 7.8 Hz, ArH), 4.25 (3H, s, $N-CH_3$).

Analysis calculated for $C_{32}H_{24}Cl_2N_4O_2S_2$ requires:
C, 60.8; H, 3.8; N, 8.9%.
Found: C, 60.4; H, 4.0; N, 8.8%.

Compound 75 of Table 1

1,4-Dimethyl-2-indolinethione [IX: R_1 = 4-Me, R_3 = Me] (81%); mp 160-162°C.

Analysis calculated for $C_{10}H_{11}NS$ requires:
C, 67.8; H, 6.3; N, 7.9; S, 18.1%
Found: C, 68.0; H, 6.4; N, 8.0; S, 18.3%

was prepared by the method given for Compound **77** (below).

Reaction of this with phenyl isocyanate gave bis[N-phenyl 1,4-dimethylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 4-CH_3, R_2 = CONHPh, R_3 = Me] (**75**); mp 237-239°C.

^{1}H NMR ($CDCl_3$): δ 8.30 (1H, br s, CONH), 7.14 (1H, dd, J = 7.3, 7.3 hz, H-6), 7.04-6.86 (7H, m, H-5,7 and CONHPh), 3.69 (3H, s, NCH_3), 2,47 (3H, s, 4-CH_3).

^{13}C NMR ($CDCl_3$): δ 164.57 (CONHPh), 138.59, 137.62, 131.51 (3xs), 128.62 (d), 127.23 (s), 125.11 (d), 124.15 (s), 123.94, 122.62 (2xd), 122.10 (s), 119.61, 107.91 (2xs), 30.26 (NCH_3), 19.66 (4-CH_3).

Analysis calculated for $C_{34}H_{30}N_4O_2S_2$ requires:
C, 69.1; H, 5.1; N, 9.5; S, 10.9%.
Found: C, 69.1; H, 5.1; N, 9.7; S, 11.0%.

Compound 76 of Table 1

1,5-Dimethyl-2-indolinethione [IX: R_1 = 5-Me, R_3 = Me]; mp 143-145°C (Bull. Fr. 1987:181 reports mp 132-133°C) was prepared by the method given for Compound **77** (below). Reaction of this with phenyl isocyanate gave bis[N-phenyl 1,5-dimethylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 5-CH_3, R_2 = CONHPh, R_3 = Me] (**76**); mp 231-234°C.

^{1}H NMR ($CDCl_3$): δ 8.24 (1H, br s, CONH), 7.78 (1H, br, H-4), 7.19-7.13 (4H, m, CONHPh), 7.05-6.90 (3H, m, H-6,7 and CONHPh), 3.71 (3H, s, NCH_3), 2.36 (3H, s, 5-CH_3).

^{13}C NMR ($CDCl_3$): δ 161.75 (CONH), 138.00, 137.10, 131.77, 129.01 (4xs), 128.53, 127.37 (2xd), 126.35 (s), 123.40, 121.33, 119.85, 109.85 (4xd), 30.32 (NCH_3), 21.57 (5-CH_3).

Analysis calculated for $C_{34}H_{30}N_4O_2S_2$ requires:
C, 69.1; H, 5.1; N, 9.5; S, 10.9%.
Found: C, 69.4; H, 5.2; N, 9.6; S, 11.2%.

Compound 77 of Table 1

A mixture of 2,5-dimethylaniline (27.4 g, 0.2 mol) and benzotriazole (23.8 g, 0.2 mol) in EtOH (300 mL) was stirred at 20°C as 37% aqueous formaldehyde (16.1 g, 0.2 mol) was added gradually. After 30 minutes, the white solid which precipitated was collected and washed with EtOH to give N-(1-benzotriazolylmethyl)-2,5-dimethylaniline (33.9 g, 67% yield); mp (EtOH) 147-149°C.

^{1}H NMR ($CDCl_3$): δ 6.85-8.10 (7H, m, ArH), 6.56 (minor isomer) and 6.13 (major isomer) (2H, 2xm, CH_2), 5.08 (minor) and 4.70 (major) (1H, 2xm, NH), 2.24 (3H, s, CH_3), and 2.12 (3H, s, CH_3).

Analysis calculated for $C_{15}H_{16}N_4$ requires:
C, 70.6; H, 5.9; N, 23.5%.
Found: C, 71.5; H, 6.3; N, 22.1%.

A suspension of this compound (33 g, 0.13 mol) and $NaBH_4$ (5 g) in dioxane (400 mL) was heated under reflux for 5 hours, and the solution was concentrated. After cooling, water was added and the resulting mixture was extracted with EtOAc. The organic layer was washed twice with aqueous K_2CO_3 and water, and dried (Na_2SO_4). Removal of the solvent gave N,2,5-trimethylaniline (17.6 g, 99% yield) as an oil, which was used directly.

^{1}H NMR ($CDCl_3$): δ 6.93 (1H, d, J = 7.4 Hz, H-3), 6.49 (1H, d, J = 7.6 Hz, H-4), 6.44 (1H, s, H-6), 3.72, (1H, s, NH), 2.88 (3H, s, NCH_3), 2.31 (3H, s, CH_3), and 2.09 (3H, s, CH_3).

A solution of 2,4,6-trimethylaniline (6.86 g, 5 mmol) in dry THF (100 mL) under an atmosphere of N_2 was cooled to -78°C and n-butyllithium (21 mL, 2.5 M solution in hexanes) was added dropwise. The mixture was allowed to warm to 0°C, and dry CO_2 gas was bubbled in for 2-3 minutes. The excess CO_2 was removed under

vacuum, and after the addition of further THF to replace that lost by evaporation, the solution was recooled to -78°C. n-Butyllithium (22 mL, 2.5 M solution in hexanes) was again added dropwise, and the temperature was then allowed to rise slowly to -10°C where a deep red colored solution was obtained. After a further 30 minutes at that temperature, the mixture was again recooled to -78°C and CO_2 gas was bubbled in until the red color disappeared. The reaction mixture was allowed to warm to 20°C, and after removal of the solvent, 0.1 M HCL (50 mL) was added to initiate both deprotection of the nitrogen and ring-closure. The resulting mixture was extracted with EtOAc, and this was then washed successively with 0.1 M HCl, water, and dilute aqueous Na_2CO_3. After drying (Na_2SO_4), the solvent was removed under vacuum, to leave an oil which was purified by chromatography on Al_2O_3 to give 1,6-dimethyl-2-indolinone (3.37 g, 42% yield) [VII: R_1 = 6-Me; R_3 = Me]; mp (hexane) 94.5-96°C. ^1H NMR ($CDCl_3$): δ 7.11 (2H, d, J = 7.5 Hz, H-4), 6.85 (2H, d, J = 7.5 Hz, H-5), 6.65 (1H, s, H-7), 3.47 (2H, s, CH_2), 3.19 (3H, s, 1-CH_3), and 2.38 (3H, s, 6-CH_3).

Analysis calculated for $C_{10}H_{11}NO$ requires:
C, 74.5; H, 6.9; N, 8.7%.
Found: C, 74.5; H, 6.6; N, 8.7%.

Thiation of this with P_2S_5 as above gave 1,6-dimethyl-2-indolinethione [IX: R_1 = 6-Me, R_3 = Me]; mp 141-143°C.

Analysis calculated for $C_{10}H_{11}NS$ requires:
C, 67.8; H, 6.3; N, 7.9; S, 18.1%.
Found: C, 67.6; H, 6.5; N, 8.2; S, 18.0%.

This was reacted with phenyl isocyanate as above to give bis[N-phenyl 1,6-dimethylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 6-CH_3, R_2 = CONHPh, R_3 = Me] (**77**); mp 192-195°C.

^{1}H NMR ($CDCl_3$): δ 8.16 (1H, br s, CONH), 7.85 (1H, d, J = 8.3 Hz, H-4), 7.10 (4H, br, CONH<u>Ph</u>, 6.98 (1H, m, CONH<u>Ph</u>), 6.87 (1H, d, J = 8.3 Hz, H-5), 6.73 (1H, br, H-7), 3.71 (3H, s, NCH_3), 2.35 (3H, s, 6-CH_3).

^{13}C NMR ($CDCl_3$): δ 161.49 (CONH), 139.05, 137.98, 135.63 (3xs), 128.44 (d), 126.10 (s), 124.28 (d), 124.06 (s), 123.17, 121.61, 119.21, 109.85 (4xd), 30.17 (NCH_3), 21.98 (6-CH_3).

Analysis calculated for $C_{34}H_{30}N_4O_2S_2$ requires:
C, 69.1; H, 5.1; N, 9.5; S, 10.9%.
Found: C, 68.9; H, 5.2; N, 9.6; S, 11.0%.

<u>Compound 78 of Table 1</u>

Similarly was prepared 1,7-dimethyl-2-indolinethione [IX: R_1 = 7-Me, R_3 = Me]; mp 138-9°C.

Analysis calculated for $C_{10}H_{11}NS$ requires:
C, 67.8; H. 6.3; N, 7.9; S, 18.1%.
Found: C, 67.6; H, 6.2; N, 8.0; S, 18.1%.

Reaction of this with phenyl isocyanate gave bis[N-phenyl 1,7-dimethylindolyl-3-carboxamide-(2)]-disulfide [V: R_1 = 7-CH_3, R_2 = CONHPh, R_3 = Me] (**78**); mp 221-223°C.

^{1}H NMR ($CDCl_3$): δ 8.11 (1H, br s, CONH), 7.83 (1H, J = 8.1 Hz, H-4), 7.15-7.07 (4H, m, CONH<u>Ph</u>), 6.99 (1H, m, CONH<u>Ph</u>), 6.94 (1H, dd, J = 8.1, 8.1 Hz, H-5), 6.85 (1H, d, J = 8.1 Hz, H-6), 4.07 (3H, s, NCH_3), 2.44 (3H, s, 7-CH_3).

^{13}C NMR ($CDCl_3$): δ 161.67 (CONH), 137.95, 137.86 (2xs), 128.55, 128.31 (2xd), 126.85 (s), 123.57, 122.10

(2xd), 121.77 (s), 119.72, 119.21 (2xd), 33.36 (NCH_3), 20.23 (7-CH_3).

Analysis calculated for $C_{34}H_{30}N_4O_2S_2$ requires:
C, 69.1; H, 5.1; N, 9.5; S, 10.9%.
Found: C, 69.1; H, 5.2; N, 9.7; S, 11.0%.

Compound 79 of Table 1

Similarly was prepared, from 4-methoxy-1-methyl-2-indolinethione [IX: R_1 = 4-OMe, R_3 = Me]; mp 141-144°C (US Patent 5,030,646 records mp 126-128°C) and phenyl isocyanate, bis[N-phenyl 4-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 4-OCH_3, R_2 = CONHPh, R_3 = Me] (**79**); mp 225-228°C.

^1H NMR ($CDCl_3$): δ 8.85 (1H, br s, CONH), 7.25-7.06 (5H, m, H-6 and CONHPh), 6.98 (1H, m, CONHPh), 6.82 (1H, d, J = 8.3 Hz, H-7), 6.36 (1H, d, J = 7.8 Hz, H-5), 3.76 (3H, s, OCH_3), 3.69 (3H, s, NCH_3).

^{13}C NMR ($CDCl_3$): 162.36 (CONH), 152.70, 139.39, 138.73, 130.20 (4xs), 128.54, 125.39, 123.08 (3xs), 130.20 (s), 128.54, 125.39, 123.08 (3xd), 19.96 (s), 119.19 (d), 114.66 (s), 103.67, 101.55 (2xd), 22.58 (OCH_3), 30.48 (NCH_3).

Analysis calculated for $C_{34}H_{30}N_4O_4S_2$ requires:
C, 65.6; H, 4.9; N, 9.0; S, 10.3%.
Found: C, 65.7; H, 4.9; N, 9.2; S, 10.2%.

Compound 80 of Table 1

Similarly was prepared, from 5-methoxy-1-methyl-2-indolinethione [IX: R_1 = 5-OMe, R_3 = Me]; mp 148-150°C (US Patent 5,030,646 records mp 142-144°C) and phenyl isocyanate, bis[N-phenyl 5-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide

[V: R_1 = 5-OCH_3, R_2 = CONHPh, R_3 = Me] (**80**);
mp 161-164°C.
^1H NMR ($CDCl_3$): δ 8.41 (1H, br s, CONH), 7.55 (d, J = 1.8 Hz, H-4), 7.18 (4H, m, CONHPh), 7.00 (2H, m, H-6 and CONHPh), 6.89 (1H, d, J = 7.4 Hz, H-7), 3.82 (3H, s, OCH_3), 3.68 (3H, s, NCH_3).
^{13}C NMR ($CDCl_3$): δ 161.80 (CONH), 155.94, 137.87, 134.07 (3xs), 128.71, 123.68, 119.50, 117.48, 111.10, 102.29 (6xd), 55.63 (OCH_3), 30.47 (NCH_3).
Analysis calculated for $C_{34}H_{30}N_4O_4S_2$ requires:
C, 65.6; H, 4.9; N, 9.0; S, 10.3%.
Found: C, 65.3; H, 5.1; N, 9.2; S, 10.4%.

Compound **81** of Table 1

Similarly was prepared, from 6-methoxy-1-methyl-2-indolinethione [IX: R_1 = 6-OMe, R_3 = Me]; mp 133-136°C (US Patent 5,030,646 records mp 135-136°C) and phenyl isocyanate, bis[N-phenyl 6-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 6-OCH_3, R_2 = CONHPh, R_3 = Me] (**81**); mp 197-200°C.
^1H NMR ($CDCl_3$): δ 8.19 (1H, br s, CONH), 7.91 (1H, d, J = 8.9 Hz, H-4), 7.12 (4H, br, CONHPh), 6.97 (1H, m, CONHPh), 6.71 (1H, d, J = 8.9 Hz, H-5), 6.25 (1H, br, H-7), 3.74 (3H, s, OCH_3), 3.70 (3H, s, NCH_3).
^{13}C NMR ($CDCl_3$): δ 161.37 (CONH), 158.75, 139.82, 138.04, 128.65 (4xs), 128.50, 123.30, 123.12, (3xd), 120.64, 120.26 (2xs), 119.10, 113.22, 98.02 (3xd), 55.26 (OCH_3), 30.21 (NCH_3).
Analysis calculated for $C_{34}H_{30}N_4O_4S_2$ requires:
C, 65.6; H, 4.9; N, 9.0; S, 10.3%.
Found: C, 65.5; H, 4.8; N, 9.2; S, 10.4%.

Compound 82 of Table 1

Similarly was prepared, from 7-methoxy-1-methyl-2-indolinethione [IX: R_1 = 7-OMe, R_3 = Me]; mp 124-126°C (US Patent 5,030,646 records mp 114-116°C) and phenyl isocyanate, bis[N-phenyl 7-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = 7-OCH_3, R_2 = CONHPh, R_3 = Me] (**82**); mp 205-208°C.

^1H NMR ($CDCl_3$): δ 8.14 (1H, br s, CONH), 7.57 (1H, d, J = 8.2 Hz, H-4), 7.13 (4H, m, CONHPh), 6.96 (1H, m, CPNHPh), 6.93 (1H, dd, J = 8.2, 8.2 Hz, H-5), 6.48 (1H, d, J = 8.2 Hz, H-6), 4.12 (3H, s, OCH_3), 3.73 (3H, s, NCH_3).

^{13}C NMR ($CDCl_3$): δ 161.72 (CONH), 147.12, 137.99, 129.08 (3xs), 128.45 (d), 128.01 (s), 123.27, 122.35, 119.33, 114.13, 105.35 (5xd), 55.22 (OCH_3), 33.73 (NCH_3).

Analysis calculated for $C_{34}H_{30}N_4O_4S_2$ requires:
C, 65.6; H, 4.9; N, 9.0; S, 10.3%.
Found: C, 64.9; H, 5.0; N, 9.0; S, 10.4%.

Compound 84 of Table 1

A solution of 3-(methylthio)-5-(trifluoromethyl)-oxindole (Gassman PG, Cue BW, Luh T-Y, J. Org. Chem. 1977;42:1344-1348) (10 g, 40 mmol) in AcOH (100 mL) was heated under reflux with Zn dust (13.3 g, 0.2 mol) for 1 hour. The mixture was cooled and filtered, and the precipitate was washed with AcOH. The combined filtrates were evaporated under reduced pressure, and the residue was diluted with 1 M aqueous ammonia to give 5-trifluoromethyloxindole [VII: R_1 = 5-CF_3, R_3 = H] (7.22 g, 90%); mp (aqueous EtOH) 188.5-191°C (lit. [Hardtmann GE, USP 4,160,032; Chem. Abstr. 1979;91:P107890w]; mp 188-189°C).

^{1}H NMR ($CDCl_3$): δ 8.74 (1H, s, NH), 7.52 (1H, d, J = 8.2 Hz, H-6), 7.49 (1H, s, H-4), 6,97 (1H, d, J = 8.2 Hz, H-7), 3.61 (2H, s, CH_2).

A suspension of the above oxindole (5.03 g, 25 mmol) in water (100 mL) containing NaOH (1.5 g) was treated with Me_2SO_4 (4.7 g, 37 mmol). The mixture was warmed to 100°C for 10 minutes, cooled, a further portion of Me_2SO_4 and NaOH added, and warmed again briefly. After thorough cooling, the solid was collected and chromatographed on alumina. Elution with CH_2Cl_2/hexane (7:3) gave 1-methyl-5-(trifluoromethyl)-oxindole [VII: R_1 = 5-CF_3, R_3 = Me] (3.5 g, 65%); mp (hexane) 127.5-129°C.

^{1}H NMR ($CDCl_3$): δ 7.58 (1H, d, J = 8.2 Hz, H-6), 7.50 (1H, s, H-4), 6.89 (1H, d, J = 8.2 Hz, H-7), 3.58 (2H, s, CH_2), 3.25 (3H, s, CH_3).

Analysis calculated for $C_{10}H_8F_3NO$ requires:
C, 55.8; H, 3.8; N. 6.5%.
Found: C, 55.5; H, 3.8; N, 6.5%.

Reaction of this compound with P_2S_5 as above gave 1-methyl-5-(trifluoromethyl)-2-indolinethione [IX: R_1 = 5-CF_3, R_3 = Me] (96% yield); mp 124.5-126°C.

^{1}H NMR ($CDCl_3$): δ 7.63 (1H, dd, J = 8.3, 0.8 Hz, H-6), 7.54 (1H, d, J = 0.8 Hz, H-4), 7.03 (1H, d, J = 8.3 Hz, H-7), 4.15 (2H, s, C-3), 3.64 (3H, s, N-CH_3).

^{13}C NMR: δ 202.28 (C-2), 149.34 (s), 129.60 (s), 126.54 (J = 32.5 Hz, C-5), 125.9 (J = 4.0 Hz), 124.21 (J = 271.9 Hz) (CF_3), 121.00 (J = 3.8 Hz), 109.28 (d), 48.75 (C-3), 31.35 (N-CH_3).

Analysis calculated for ($C_{10}H_8F_3NS$) requires:
C, 51.9; H, 3.5; N, 6.3; S, 14.1%.
Found: C, 52.0; H, 3.7; N, 6.3; S, 14.1%.

Reaction of this with phenyl isocyanate as above gave 2,2-dithiobis[*N*-phenyl-1-methyl-5-(trifluoro-

methyl)indolyl-3-carboxamide] (84) [V: R_1 = 5-CF_3, R_2 = CONHPh, R_3 = Me] (71% yield); mp 214-216°C.
^{1}H NMR ($(CD_3)_2SO$): δ 9.53 (1H, s, CONH), 8.14 (1H, br s, H-4), 7.59 (1H, d, J = 8.8 Hz, H-7), 7.53 (1H, dd, J = 8.8, 1.5 Hz, H-6), 7.12-7.09 (4H, m, ArH), 6.97 (1H, m, ArH), 3.76 (3H, s, N-CH_3).
^{13}C NMR: δ 160.49 (CONH), 138.93 (s), 138.21 (s), 131.76 (s), 128.19 (d), 124.96 (J = 271.6 Hz, CF_3), 124.60 (d), 119.21 (s), 119.09 (d), 118.57 (J = 4.1 Hz), 30.46 (N-CH_3).
Analysis calculated for $C_{34}H_{24}F_6N_4O_2S_2$ requires:
C, 58.4; H, 3.5; N, 8.0; S, 9.2%.
Found: C, 58.5; H, 3.8; N, 7.9; S, 9.3%.

Compound 85 of Table 1

Methylation of 6-chlorooxindole [VII: R_1 = 6-Cl, R_3 = H] (Quallich GJ, Morrissey PM, Synthesis 1993:51-53) with Me_2SO_4/NaOH as above gave 6-chloro-1-methyloxindole [VII: R_1 = 6-Cl, R_3 = CH_3]; mp (aqueous EtOH) 119.5-122°C.
^{1}H NMR ($CDCl_3$): δ 7.15 (1H, d, J = 7.8 Hz, H-4), 7.01 (1H, dd, J = 7.8, 1.8 Hz, H-5), 6.82 (1H, d, J = 1.7 Hz, H-7), 3.49 (2H, s, CH_2), 3.19 (3H, s, CH_3).
Analysis calculated for C_9H_8ClNO requires:
C, 59.5; H, 4.4; N, 7.7%.
Found: C, 59.6; H, 4.6; N, 7.6%.

Reaction of this with P_2S_5 as above gave 6-chloro-1-methyl-2-indolinethione [IX: R_1 = 6-Cl, R_3 = Me] (87% yield); mp (EtOAc/petroleum ether) 162-165°C.
^{1}H NMR ($CDCl_3$): δ 7.20 (1H, d, J = 7.9 Hz, H-4), 7.13 (1H, dd, J = 7.9, 1.7 Hz, H-5), 6.96 (1H, d, J = 1.7 Hz, H-7), 4.06 (2H, s, H-3), 3.59 (3H, s, N-CH_3).

^{13}C NMR: δ 202.00 (C-2), 147.76 (s), 133.98 (s), 127.35 (s), 124.64 (d), 124.06 (d), 110.20 (d), 48.59 (C-3), 31.29 (N-CH_3).

Analysis calculated for $C_9H_8ClN_2SO$ requires:

C, 54.7; H, 4.1; N, 7.1; S, 16.2%.

Found: C, 54.8; H, 4.1; N, 7.0; S, 16.3%.

Reaction of this with phenyl isocyanate as above gave bis[N-phenyl 6-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide (**85**) [V: R_1 = 6-Cl, R_2 = CONHPh, R_3 = Me] (61% yield); mp 243-245°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.43 (1H, br, CONH), 7.77 (1H, d, *J* = 8.6 Hz, H-4), 7.46 (1H, d, *J* = 1.4 Hz, H-7), 7.19-7.09 (5H, m, ArH), 7.01 (1H, m, ArH), 3.67 (3H, s, N-CH_3).

^{13}C NMR: δ 160.66 (CONH), 138.29 (s), 138.04 (s), 129.87 (s), 129.41 (s), 128.15 (d), 123.94 (s), 122.91 (d), 122.37 (d), 121.70 (d), 119.20 (s), 119.12 (d), 110.69 (d), 30.22 (N-CH_3).

Analysis calculated for $C_{32}H_{24}Cl_2N_4O_2S_2$ requires:

C, 60.9; H, 3.8; N, 8.9; S, 10.2%.

Found: C, 60.9; H, 4.0; N, 8.7; S, 10.2%.

Compound **86** of Table 1

Similarly was prepared, from 1-methyl-5-nitro-2-oxindole (Robinson R, Wyler M, J. Chem. Soc. 1941:620-624), 1-methyl-5-nitro-2-indolinethione [IX: R_1 = 5-NO_2, R_3 = Me] (68% yield); mp (EtOAc/light petroleum) >330°C.

^{1}H NMR ($(CD_3)_2SO$): δ 8.28 (1H, dd, *J* = 8.7, 1.7 Hz, H-6), 8.17 (1H, d, *J* = 1.7 Hz, H-4), 7.41 (1H, d, *J* = 8.7 Hz, H-7), 4.26 (2H, s, H-3), 3.60 (3H, s, N-CH_3).

^{13}C NMR: δ 203.48 (C-2), 151.49 (s), 143.81 (s), 130.53 (s), 124.80 (d), 119.00 (d), 110.24 (d), 48.45 (C-3), 31.34 (N-CH_3).

Analysis calculated for $C_9H_8N_2SO_2$ requires:

M+ 208.0306.

Found: M+ 208.0311 (mass spectrum).

Reaction of this with phenyl isocyanate as above gave 2,2'-dithiobis[*N*-phenyl-1-methyl-5-nitroindolyl-3-carboxamide] (**86**) [V: R_1 = 5-NO_2, R_2 = CONHPh, R_3 = Me] (52% yield); mp 236-240°C (dec).

^1H NMR ($(CD_3)_2CO$): δ 9.68 (1H, br, CONH), 8.64 (1H, d, J = 1.6 Hz, H, H-4), 8.07 (1H, dd, J = 8.8, 1.6 Hz, H-6), 7.56 (1H, d, J = 8.8 Hz, H-7), 7.18-7.08 (4H, m, ArH), 6.98 (1H, t, J = 6.8 Hz, ArH), 3.79 (3H, s, N-CH_3).

^{13}C NMR: δ 160.04 (CONH) 141.96 (s), 140.17 (s), 138.22 (s), 128.24 (d), 124.35 (s), 123.09 (d), 120.25 (s), 118.90 (d), 117.76 (d), 111.64 (d), 30.70 (N-CH_3).

Analysis calculated for $C_{32}H_{24}N_6O_6S_2 \cdot 0.2H_2O$ requires:

C, 55.8; H, 4.1; N, 12.2%.

Found: C, 55.5; H, 3.9; N, 12.0%.

Analysis calculated for $C_{32}H_{25}N_6S_2O_6$ requires:

$[M + H]^+$ 653.1277.

Found: $[M + H]^+$ 653.1275 (FAB mass spectrum).

Compound 87 of Table 1

Similarly was prepared, from 5-fluoro-1-methyloxindole (Wiseman EH, Chiaini J, McManus JM, J. Med. Chem. 1973;16:131-134), 5-fluoro-1-methyl-2-indolinethione [IX: R_1 = 5-F, R_3 = Me] (93% yield); mp 155-157°C.

^1H NMR ($CDCl_3$): δ 7.11-6.99 (2H, m, H-4,6), 6.88 (1H, dd, J = 9.3, 4.2 Hz, H-7), 4.09 (2H, s, H-3), 3.61 (3H, s, N-CH_3).

^{13}C NMR: δ 200.61 (C-2), 160.49 (J = 243.6 Hz, C-5), 142.76 (s), 130.80 (J = 8.6 Hz, C-3a), 114.48 (J = 24.1 Hz), 112.13 (J = 25.1 Hz), 109.94 (J = 8.6 Hz), 48.96 (J = 1.8 Hz, C-3), 31.38 (N-CH_3).

Analysis calculated for C_9H_8FNS requires:
C, 59.7; H, 4,5; N, 7.7; S, 17.7%.
Found: C, 59.7; H, 4.6; N, 7.8; S, 17.4%.

Reaction of this with phenyl isocyanate as above gave 2,2'-dithiobis[*N*-phenyl-5-fluoro-1-methylindolyl-3-carboxamide] (**87**) [V: R_1 = 5-F, R_2 = CONHPh, R_3 = Me]) (74% yield); mp 205-207°C.

^1H NMR ($CDCl_3$): δ 8.17 (1H, br, CONH), 7.64 (1H, dd, J = 9.4, 2.0 Hz, H-4), 7.17 (4H, br d, ArH), 7.00 (1H, m, ArH), 6.95-6.88 (2H, m, ArH), 3.78 (3H, s, N-CH_3).

^{13}C NMR: δ 161.17 (CONH), 158.97 (J = 239.4 Hz, C-5), 138.02 (s), 135.71 (s), 128.69 (d), 123.69 (d), 118.87 (d), 114.66 (J = 27.1 Hz), 111.14 (J = 10.0 Hz), 106.92 (J = 25.5 Hz), 30.61 (N-CH_3).

Analysis calculated for $C_{32}H_{24}F_2N_4O_2S_2$ requires:
C, 64.2; H, 4.0; N, 9.4; S, 10.7%.
Found: C, 63.9; H, 4.2; N, 9.3; S, 10.7%.

Compound 88 of Table 1

Reduction of 5-cyano-3-methylthiooxindole (Gassman PG, Cue BW, Luh T-Y, J. Org. Chem. 1977;42:1344-1348) with Zn/AcOH as above gave 5-cyanooxindole [VII: R_1 = 5-CN; R_3 = H] (89% yield); mp (aqueous EtOH) 249°C (dec) (lit. [Gassman PG, Gilbert DP, Luh T-Y, JOC 1977;42:1340-1344]; mp 249-251°C). Methylation of this with Me_2SO_4/NaOH as above gave 5-cyano-1-methyloxindole [VII: R_1 = 5-CN, R_3 = H] (53% yield); mp (hexane) 201-203°C.

^{1}H NMR ($CDCl_3$): δ 7.63 (1H, dd, J = 8.1, 1.1 Hz, H-6), 7.51 (1H, d, J = 1.1 Hz, H-4), 6.90 (1H, d, J = 8.1 Hz, H-7), 3.57 (2H, s, CH_2), 3.25 (3H, s, CH_3).

Analysis calculated for $C_{10}H_8N_2O$ requires:

C, 69.8; H, 4.7; N, 16.3%.

Found: C, 70.2; H, 4.64; N, 16.7%.

Reaction of the above compound with P_2S_5 gave 5-cyano-1-methyl-2-indolinethione [IX: R_1 = 5-CN, R_3 = Me] (41% yield); mp 185-187°C.

^{1}H NMR ($(CD_3)_2SO$): δ 7.87 (1H, br d, J = 8.3 Hz, H-6), 7.76 (1H, br s, H-4), 7.41 (1H, d, J = 8.3 Hz, H-7), 4.22 (2H, s, H-3), 3.58 (3H, s, N-CH_3).

^{13}C NMR: δ 202.34 (C-2), 149.78 (s), 133.05 (d), 130.42 (s), 126.92 (d), 119.05 (s), 110.98 (d), 48.20 (C-3), 31.11 (N-CH_3).

Analysis calculated for $C_{10}H_8N_2S \cdot 0.5H_2O$ requires:

C, 60.7; H, 4.6; N, 14.2%.

Found: C, 61.3; H, 4.1; N, 14.4%.

Reaction of this with phenyl isocyanate as above gave 2,2'-dithiobis[*N*-phenyl-5-cyano-1-methylindolyl-3-carboxamide] (88) [V: R_1 = 5-CN, R_2 = CONHPh, R_3 = Me] (47% yield); mp 221-224°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.51 (1H, s, CONH), 8.18 (1H, br s, H-4), 7.60-7.48 (2H, m, H-6,7), 7.20-7.06 (4H, m, ArH), 7.00 (1H, br s, ArH), 3.75 (3H, s, N-CH_3).

^{13}C NMR: δ 160.21 (CONH), 138.97 (s), 138.26 (s), 132.74 (C-5), 128.77 (s), 128.27 (d), 126.52 (d), 124.72 (s), 123.14 (d), 119.80 (s), 119.11 (d), 118.87 (s), 112.29 (d), 103.53 (CN), 30.46 (N-CH_3).

Analysis calculated for $C_{34}H_{24}N_6O_2S_2 \cdot 0.5H_2O$ requires:

C, 65.7; H, 4.1; N, 13.5; S, 10.3%.

Found: C, 65.6; H, 4.0; N, 13.5; S, 10.6%.

Compound 89 of Table 1

Similarly was prepared, from 5-bromo-1-methyl-2-indolinethione [IX: R_1 = 5-Br, R_3 = Me]; mp 137-139°C, (Baudin J-B, Julia SA, Lorne R, Bull. Soc. Chim. France 1987:181 records mp 126-127°C) and phenyl isocyanate as above, 2,2'-dithiobis[*N*-phenyl-5-bromo-1-methylindolyl-3-carboxamide] (**89**) [V: R_1 = 5-Br, R_2 = CONHPh, R_3 = Me] (68% yield); mp 219-221°C.

^{1}H NMR ($CDCl_3$): δ 8.14 (1H, br, CONH), 8.10 (1H, d, J = 1.6 Hz, H-4), 7.21-7.12 (5H, m, ArH), 7.01 (1H, m, ArH), 6.83 (1H, br d, J = 8.2 Hz, ArH), 3.73 (3H, s, N-CH_3).

^{13}C NMR: δ 161.04 (CONH), 137.68 (s), 137.00 (s), 128.75 (d), 128.60 (d) 127.13 (s), 124.29 (d), 123.78 (d), 118.82 (d), 115.92 (s), 111.46 (d), 30.48 (N-CH_3).

Analysis calculated for $C_{32}H_{24}Br_2N_4O_2S_2$ requires:
C, 53.3; H, 3.4; N, 7.8; S, 8.9%.
Found: C, 53.1; H, 3.5; N, 7.7; S, 8.9%.

Compound 90 of Table 1

A solution of 4-methoxy-1-methyl-2-oxindole [VII: R_1 = 4-OMe, R_3 = Me] (1.20 g, 6.77 mmol) in 48% HBr/glacial AcOH (40 mL) was heated under reflux for 6 hours, then poured into water. The precipitate of crude phenol was filtered off, washed well with water and dried, then acetylated with Ac_2O/pyridine for 1 hour at 20°C. Solvents were removed under reduced pressure, and the residue was partitioned between EtOAc and 3N HCl. Chromatography of the organic residue on silica gel, eluting with EtOAc/petroleum ether gave 4-acetoxy-1-methyl-2-oxindole [VII: R_1 = 4-OAc, R_3 = Me] (75% yield); mp 109-111°C.

^{1}H NMR ($CDCl_3$): δ 7.30 (1H, dd, J = 8.2, 7.7 Hz, H-6), 6.78 (1H, d, J = 8.2 Hz, H-7), 6.71 (1H, d, J = 7.7 Hz, H-5), 3.41 (2H, s, H-3), 3.22 (3H, s, N-CH_3), 2.32 (3H, s, $OCOCH_3$).

^{13}C NMR: δ 174.26 (C-2), 168.30 ($O\underline{C}OCH_3$), 164.71 (s), 146.58 (s), 129.12, 116.62 (s), 115.83 (d), 105.90 (d), 33.74 (C-3), 26.51 (N-CH_3), 20.83 ($COO\underline{C}H_3$).

Analysis calculated for $C_{11}H_{11}NO_3$ requires:
C, 64.4; H, 5.4; N, 6.8%.
Found: C, 64.3; H, 5.4; N, 7.0%.

Reaction of this with P_2S_5 as above gave 4-acetoxy-1-methyl-2-indolinethione [IX: R_1 = 4-OAc, R_3 = Me] (94% yield); mp 156°C.

^{1}H NMR ($CDCl_3$): δ 7.35 (1H, dd, J = 8.2, 7.9 Hz, H-6), 6.90 (1H, d, J = 8.2 Hz, H-7), 6.86 (1H, d, J = 7.9 Hz, H-5), 4.00 (2H, s, H-3), 3.61 (3H, s, N-CH_3), 2.32 (3H, s, $OCOCH_3$).

^{13}C NMR: δ 200.75 (C-2), 168.14 ($O\underline{C}OCH_3$), 148.30 (s), 146.27 (s), 129.44 (d), 121.18 (s), 117.69 (d), 107.32 (d), 47.09 (C-3), 31.57 (N-CH_3), 20.81 ($COO\underline{C}H_3$).

Analysis calculated for $C_{11}H_{11}NO_2S$ requires:
C, 59.7; H, 5.0; N, 6.3; S, 14.5%.
Found: C, 59.4; H, 5.2; N, 6.6; S, 14.5%.

Reaction with phenyl isocyanate as above gave 2,2'-dithiobis[N-phenyl 4-acetoxy-1-methylindolyl-3-carboxamide] (90) [V: R_1 = 4-OAc, R_2 = CONHPh, R_3 = Me] (31%); mp 194°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.92 (1H, s, CONH), 7.34-7.27 (4H, m, H-5,7,2',6'), 7.14 (2H, dd, J = 7.8, 7.6 Hz, H-3',5'), 6.98 (1H, t, J = 7.8 Hz, H-5'), 6.89 (1H, dd, J = 8.0, 7.8 Hz, H-5), 3.66 (3H, s, NCH_3), 1.95 (3H, s, OCH_3).

^{13}C NMR: δ 168.57 ($\underline{C}ONHPh$), 162.09 ($O\underline{C}OCH_3$), 142.91 (s), 139.20 (s), 138.75 (s), 129.01 (s), 128.38 (d),

124.56 (d), 123.14 (d), 119.23 (s), 118.38 (d), 117.70 (s), 113.94 (d), 108.70 (d), 30.39 (N-CH_3), 20.32 (COO$\underline{C}H_3$).

Analysis calculated for $C_{36}H_{30}N_4O_6S_2$ requires:

679.1685.

Found: $[M + H]^+$ 679.1705 (FABMS).

Compound 91 of Table 1

Similar demethylation/acetylation of 5-methoxy-1-methyl-2-oxindole [VII: R_1 = 5-OMe, R_3 = Me] gave 5-acetoxy-1-methyl-2-oxindole [VII: R_1 = 5-OAc, R_3 = Me] (70% yield); mp (EtOAc/petroleum ether) 104-106°C.

^1H NMR ($CDCl_3$): δ 7.01 (1H, br s, H-4), 7.00 (1H, dd, J = 9.1, 2.4 Hz, H-6), 3.53 (2H, s, H-3), 3.20 (3H, s, N-CH_3), 2.30 (3H, s, OCOCH_3).

^{13}C NMR: δ 174.79 (C-2), 169.96 (O$\underline{C}$OCH_3), 146.08 (s), 142.96 (s), 125.50 (s), 120.84 (d), 118.54 (d), 108.25 (d), 35.89 (C-3), 26.30 (N-CH_3), 21.04 (OCO$\underline{C}H_3$).

Analysis calculated for $C_{11}H_{11}NO_3$ requires:

C, 64.4; H, 5.4; N, 6.8%.

Found: C, 64.4; H, 5.4; N, 6.8%.

Reaction of this with P_2S_5 as above gave 5-acetoxy-1-methyl-2-indolinethione [IX: R_1 = 5-OAc, R_3 = Me] (86% yield); mp 134-135.5°C.

^1H NMR ($CDCl_3$): δ 7.06 (2H, br s, H-4,6), 6.93 (1H, d, J = 8.6 Hz, H-7), 4.08 (2H, s, H-3), 3.60 (3H, s, N-CH_3), 2.31 (3H, s, OCOCH_3).

^{13}C NMR: δ 200.86 (C-2), 169.62 (O$\underline{C}$OCH_3) 147.62 (s), 144.14 (s), 130.10 (s), 120.97 (d), 117.99 (d), 109.62 (d), 48.79 (C-3), 31.24 (N-CH_3), 20.94 (OCO$\underline{C}H_3$).

Analysis calculated for $C_{11}H_{11}NO_2S$ requires:

C, 59.7; H, 5.0; N, 6.3; S, 14.5%.

Found: C, 59.6; H, 5.2; N, 6.2; S, 14.6%.

Reaction with phenyl isocyanate as above gave 2,2'-dithiobis[*N*-phenyl-5-acetoxy-1-methylindolyl-3-carboxamide] (**91**) [V: R_1 = 5-OAc, R_2 = CONHPh, R_3 = Me], (45% yield); mp 147-150°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.60 (1H, br, CONH), 7.54 (1H, d, J = 1.9 Hz, H-4), 7.42 (1H, d, J = 8.9 Hz, H-7), 7.23 (2H, d, J = 7.8 Hz, H-2',6'), 7.17 (2H, dd, J = 7.8, 7.1 Hz, H-3',5'), 7.06 (1H, dd, J = 8.9, 1.9 Hz, H-6), 6.98 (1H, t, J = 7.1 Hz, H-4), 3.66 (3H, s, NCH_3), 2.29 (3H, s, $OCOCH_3$).

^{13}H NMR: δ 169.52 (CONH), 161.18 ($O\underline{C}OCH_3$), 145.27 (s), 138.49 (s), 135.41 (s), 128.31 (d), 125.46 (s), 122.94 (d), 119.15 (d), 112.82 (d), 111.43 (d), 30.26, (N-CH_3), 20.80 ($OCO\underline{C}H_3$).

Analysis calculated for $C_{36}H_{30}N_4O_6S_2 \cdot 0.5H_2O$ requires:
C, 62.9; H, 4.5; N, 8.2; S, 9.3%.
Found: C, 63.1; H, 4.6; N, 8.2; S, 9.5%.

Compound **92** of Table 1

A stirred suspension of the 5-acetoxydisulfide (**91**) (0.25 g, 0.37 mmol) in MeOH (15 mL) was treated with $NaBH_4$ (0.05 g, 1.32 mmol) at 20°C for 10 minutes. Aqueous 3N KOH (2 mL) was then added, and after a further 15 minutes the solution was diluted with water and extracted with CH_2Cl_2. The resulting oil was immediately dissolved in MeOH (3 mL) and mixed with H_2O_2 (0.10 mL of 35%). The solution was chilled at -30°C for 48 hours and then filtered to yield 2,2'-dithiobis(*N*-phenyl-5-hydroxy-1-methylindole-3-carboxamide) (**92**) [V: R_1 = 5-OH, R_2 = CONHPh, R_3 = Me] (41 mg, 19%); mp 185-187°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.50 (1H, s, CONH), 9.15 (1H, br, OH), 7.32 (2H, d, J = 7.8 Hz, H-2',6'), 7.27 (1H, d, J = 8.9 Hz, H-7), 7.19 (1H, d, J = 2.3 Hz, H-4), 7.18

(2H, dd, J = 7.8, 7.4 Hz, H-3',5'), 6.99 (1H, t, J = 7.4 Hz, H-4'), 6.83 (1H, dd, J = 8.9, 2.3 Hz, H-6), 3.51 (3H, s, N-CH_3).

Analysis calculated for $C_{32}H_{26}N_4O_4S_2 \cdot H_2O$ requires:

C, 64.6; H, 4.4; N, 9.4%.

Found: C, 62.7; H, 4.6; N, 9.1%.

Compound 93 of Table 1

Similar demethylation/acetylation of 6-methoxy-1-methyl-2-oxindole [VII: R_1 = 6-OMe, R_3 = Me] gave 6-acetoxy-1-methyl-2-oxindole [VII: R_1 = 6-OAc, R_3 = Me] (81% yield); mp 119-121°C.

^{1}H NMR ($CDCl_3$): δ 7.22 (1H, d, J = 7.9 Hz, H-4), 6.74 (1H, dd, J = 7.9, 2.1 Hz, H-5), 6.59 (1H, d, J = 2.1 Hz, H-7), 3.49 (2H, s, H-3), 3.18 (3H, s, N-CH_3), 2.31 (3H, s, $OCOCH_3$).

^{13}C NMR: δ 175.28 (C-2), 169.57 ($O\underline{C}OCH_3$), 150.74 (s), 146.23 (s), 124.83 (d), 121.81 (s), 115.00 (d), 102.68 (d), 35.33 (C-3), 26.27 (N-CH_3), 21.09 ($OCO\underline{C}H_3$).

Analysis calculated for $C_{11}H_{11}NO_3$ requires:

C, 64.4; H, 5.4; N, 6.8%.

Found: C, 64.5; H, 5.5; N, 6.9%.

Reaction of this with P_2S_5 as above gave 6-acetoxy-1-methyl-2-indolinethione [IX: R_1 = 6-OAc, R_3 = Me] (91% yield); mp 131-133°C.

^{1}H NMR: δ ($CDCl_3$) 7.27 (1H, d, J = 8.0 Hz, H-4), 6.87 (1H, dd, J = 8.0, 1.9 Hz, H-5), 6.75 (1H, d, J = 1.9 Hz, H-7), 4.08 (2H, s, H-3), 3.58 (s, N-CH_3), 2.33 (3H, s, $OCOCH_3$).

^{13}C NMR: δ 202.18 (C-2), 169.44 ($O\underline{C}OCH_3$), 150.80 (s), 147.57 (s), 126.38 (s), 124.32 (d), 117.05 (d), 104.06 (d), 48.62 (C-3), 31.33 (N-CH_3), 21.09 ($OCO\underline{C}H_3$).

Analysis calculated for $C_{11}H_{11}NO_2S$ requires:
C, 59.7; H, 5.0; N, 6.3; S, 14.5%.
Found: C, 59.4; H, 5.2; N, 6.1; S, 14.3%.

Reaction with phenyl isocyanate as above gave 2,2'-dithiobis[*N*-phenyl-6-acetoxy-1-methylindolyl-3-carboxamide] (**93**) [V: R_1 = 6-OAc, R_2 = CONHPh, R_3 = Me] (53%); mp 219-222°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.71 (1H, br s, CONH), 7.78 (1H, d, J = 8.7 Hz, H-4), 7.27 (3H, m, H-2',6'), 7.18 (2H, dd, J = 8.2, 7.3 Hz, H-3',5'), 6.99 (1H, t, J = 7.3 Hz, H-4'), 6.95 (1H, dd, J = 8.7, 1.8 Hz, H-5), 3.60 (3H, s, NCH_3), 2.32 (3H, s, $OCOCH_3$).

^{13}C NMR: δ 169.31 (CONHPh), 161.23 ($OCOCH_3$), 147.99 (s), 138.54 (s), 137.66 (s), 128.29 (d), 123.13 (s), 122.98 (d), 121.48 (d), 119.38 (d), 118.73 (s), 116.34 (d), 103.76 (d), 30.17 ($N-CH_3$), 20.81 ($OCOCH_3$).

Analysis calculated for $C_{36}H_{30}N_4O_6S_2$ requires:
C, 63.7; H. 4.5; N, 8.3; S, 9.4%.
Found: C, 63.7; H, 4.4; N, 8.2; S, 9.8%.

Compound **94** of Table 1

Similar treatment of the 6-acetoxydisulfide (**93**) gave 2,2'-dithiobis(6-hydroxy-1-methyl-*N*-phenyl-1H-indole-3-carboxamide) (**94**) [V: R_1 = 6-OH, R_2 = CONHPh, R_3 = Me]; mp 185-187°C (dec).

^{1}H NMR ($(CD_3)_2SO$): δ 10.01, 9.43 (2H, 2s, OH and CONH), 7.76 (1H, d, J = 7.9 Hz, H-4), 7.35 (2H, d, J = 7.6 Hz, H-2',6'), 7.31 (1H, d, J = 2.2 Hz, H-7), 7.10 (2H, dd, J = 7.6, 7.4 Hz, H-3',5'), 6.95 (1H, t, J = 7.4 Hz, H-4'), 6.71 (1H, dd, J = 7.9, 2.2 Hz, H-5), 3.58 (3H, s, NCH_3).

Analysis calculated for $C_{32}H_{26}N_4O_4S_2$ requires:
595.1474.
Found: $[M + H]^+$ 595.1483 (FABMS).

Compound 95 of Table 1

Similar demethylation/acetylation of 7-methoxy-1-methyl-2-oxindole [VII: R_1 = 7-OMe, R_3 = Me] gave 7-acetoxy-1-methyl-2-oxindole [VII: R_1 = 7-OAc, R_3 = Me] (68% yield); mp 95-97°C.

^{1}H NMR ($CDCl_3$): δ 7.12 (1H, dd, J = 7.1, 1.0 Hz, H-6), 7.01 (1H, dd, J = 8.4, 7.1 Hz, H-5), 6.96 (1H, dd, J = 8.4, 1.0 Hz, H-4), 3.54 (2H, s, H-3), 3.34 (3H, s, N-CH_3), 2.35 (3H, s, OCOCH_3).

^{13}C NMR: δ 174.88 (C-2), 169.57 (O$\underline{C}$OCH$_3$), 136.11 (s), 134.24 (s), 126.73 (s), 123.02 (d), 122.60 (d), 122.18 (d), 35.68 (C-3), 28.17 (N-CH_3), 20.89 (OCO$\underline{C}$H$_3$).

Analysis is calculated for $C_{11}H_{11}NO_3$ requires:

C, 64.4; H, 5.4; N, 6.8%.

Found: C, 64.5; H, 5.5; N, 6.7%.

Reaction of this with P_2S_5 as above gave 7-acetoxy-1-methyl-2-indolinethione [IX: R_1 = 7-OAc, R_3 = Me] (85% yield); mp 133-135°C.

^{1}H NMR ($CDCl_3$): δ 7.17 (1H, d, J = 7.9 Hz, H-6), 7.14 (1H, dd, J = 8.0, 7.9 Hz, H-5), 7.01 (1H, d, J = 8.0 Hz, H-4), 4.13 (2H, s, H-3), 3.78 (3H, s, N-CH_3), 2.39 (3H, s, OCOCH_3).

^{13}C NMR: δ 202.00 (C-2), 169.22 (O$\underline{C}$OCH$_3$), 137.53 (s), 134.33 (s), 131.42 (s), 124.78 (d), 123.23 (d), 121.69 (d), 49.20 (C-3), 33.67 (N-CH_3), 20.97 (OCO$\underline{C}$H$_3$).

Analysis calculated for $C_{11}H_{11}NO_2S$ requires:

C, 59.7; H, 5.0; N, 6.3; S, 14.5%.

Found: C, 59.4; H, 5.2; N, 6.2; S, 14.2%.

Reaction with phenyl isocyanate as above gave 2,2'-dithiobis[*N*-phenyl-7-acetoxy-1-methylindolyl-3-carboxamide] (95) [V: R_1 = 7-OAc, R_2 = CONHPh, R_3 = Me]; mp 212-214.5°C.

^{1}H NMR ($(CD_3)_2SO$): δ 10.28 (1H, br, CONH), 7.72 (1H, d, J = 7.8 Hz, H-4), 7.44 (2H, d, J = 7.8 Hz, H-2',6'),

7.23 (2H, dd, J = 8.1, 7.8 Hz, H-3',5'), 7.11 (1H, dd, J = 7.8, 7.7 Hz, H-5), 7.01 (2H, m, H-6, H-4'), 3.68 (3H, s, N-CH_3), 2.35 (3H, s, OCOCH_3).

^{13}C NMR: δ 169.49 (*C*ONHPh), 161.36 (*O*COCH_3), 138.75 (s), 135.92 (s), 129.43 (s), 128.80 (s), 128.43 (d), 128.0 (s), 123.13 (d), 121.21 (d), 119.35 (d), 118.50 (d), 118.16 (d), 31.84 (OCO*C*H_3), 20.68 (N-CH_3).

Analysis calculated for $C_{36}H_{30}N_4O_6S_2 \cdot 0.5H_2O$ requires:
C, 62.9; H, 4.5; N, 8.2; S, 9.3%.
Found: C, 62.9; H, 4.5; N, 7.8; S, 9.6%.

Compound 96 of Table 1

Reaction of **96** as above with $NaBH_4$ followed by 3N KOH gave, after reoxidation, 2,2'-dithiobis(*N*-phenyl-7-hydroxy-1-methylindole-3-carboxamide) (**96**) [V: R_1 = 7-OH, R_2 = CONHPh, R_3 = Me] (81% yield); mp 207°C (dec).

^{1}H NMR ($(CD_3)_2SO$): δ 9.94, 9.63 (each 1H, 2s, CONH and ArOH), 7.33 (1H, d, J = 8.0 Hz, H-2',6'), 7.23 (1H, d, J = 8.0 Hz, H-4), 7.18 (2H, dd, J = 8.0, 8.0 Hz, H-3',5'), 6.99 (1H, t, J = 8.0 Hz, H-4'), 6.91 (1H, dd, J = 8.0, 7.5 Hz, H-5), 6.65 (1H, d, J = 7.5 Hz, H-6), 3.89 (3H, s, N-CH_3).

^{13}C NMR: δ 161.89 (CONH), 144.46 (s), 138.72 (s), 128.30 (d), 127.74 (s), 127.57 (s), 122.98 (d), 121.76 (d), 119.46 (d), 119.36 (s), 119.32 (s), 111.57 (d), 108.85 (d), 32.84 (N-CH_3).

Analysis calculated for $C_{32}H_{26}N_4O_4S_2$ requires:
C, 64.3; H, 4.4; N, 9.4; S, 10.8%.
Found: C, 64.2; H, 4.4; N, 9.3; S, 10.9%.

Compound 97 of Table 1

Similarly was prepared, from 1-methyl-2-indolinethione and methyl isocyanate, bis[N-methyl 1-methylindolyl-3-carboxamide-(2)]-disulfide [V: R_1 = H, R_2 = CONHMe, R_3 = Me] (97) (18% yield); mp 162-165°C.

^{1}H NMR ($CDCl_3$): δ 8.07 (1H, d, J = 8.0 Hz, H-4), 7.40-7.20 (3H, m, H-5, H-6, H-7), 6.31 (1H, br, CONH), 3.82 (3H, s, NCH_3), 2.13 (3H, d, J = 5.0 Hz, $CONHCH_3$).

^{13}C NMR ($CDCl_3$): δ 173.29 (CONH), 128.34 (s), 125.28, 122.31, 122.02, 120.0 (s), 116.5 (s), 113.2 (s), 110.06, 30.26 ($N-CH_3$), 25.68 ($CONHCH_3$).

Alternate Preparation of Compound 97 of Table 1

A mixture of 20 g (136 mmol) of 1-methyl-2-indolinone and 250 mL of dichloroethane was sealed in a 500 mL stainless steel autoclave. The reactor was cooled to less than -10°C and 60 g of phosgene was distilled into the vessel. The reactor was sealed and heated to 80°C while rocking. After 1 hour, the reactor was cooled to room temperature, vented, and purged with nitrogen. The reactor was opened and the solution was rinsed out with fresh dichloromethane. The dichloroethane solution from the rinsed reactor was concentrated to a purple solid. The solid was dissolved into 300 mL of dichloromethane and the solution was cooled in an ice bath. Into the cold solution was bubbled anhydrated methylamine at a moderate rate over a 50-minute period. The mixture was washed with water (2 x 300 mL) and brine, dried (Na_2SO_4), and concentrated to ca. 150 mL. The solution was purified by flash silica gel chromatography (7.5 x 13 cm bed) eluting with 1.6 L dichloromethane, 2 L 2%, then 2 L 5% acetone/dichloromethane, with

500 mL fractions collected. Impure early product fractions were combined, concentrated, and recrystallized from 40 mL ethanol/12 mL pet ether to give 3.04 g of 2-chloro-1-methylindole-3-N-methylcarboxamide [XXII: R_6 = H, R_7 = CH_3]; mp 148-151°C. Pure product fractions were combined and concentrated to give 16.41 g of additional product as a pale yellow solid; mp 150-151°C. Total yield = 19.45 g (64%).

Reaction of 9.30 g (41.8 mmol) of the above carboxamide was carried out with 129.5 mmol of MeSLi in 36 mL of DMA. After heating at 60°C for 7 hours, the clear amber solution was cooled in an ice bath and treated slowly with 150 mL of 5% aqueous HCl. The resultant suspension was diluted with ca. 150 mL of dichloromethane, and the mixture was stirred for 1 hour. The layers were separated, and the aqueous phase was extracted twice more. The combined organic extracts were washed with water (3 x 200 mL), then brine, dried $MgSO_4$, and concentrated to a residue that was pumped at 0.05 mm for 1 hour to leave 12.5 g of an orange solid. The solid was suspended into 100 mL of HOAc and 50 mL of water, and with vigorous stirring the suspension was treated with 12.85 g of sodium perborate. The thick suspension was stirred for ca. 30 minutes, then filtered using 10% methanol in water to aid in the transfer. The solids were washed well with water, then with ether, and air dried. Further drying at 200 mm/65°C/overnight over P_2O_5 afforded 6.38 g (70%) of pure bis[N-methyl 1-methylindolyl-3-carboxamide-(2)]disulfide (97) [V: R_2 = $CONHCH_3$]; mp 186-187°C.

Compound 98 of Table 1

Similarly was prepared, from 1-methyl-2-indolinethione and benzyl isocyanate, bis[N-benzyl 1-methylindolyl-3-carboxamide-(2)]disulfide [V: R_1 = H, R_2 = $CONHCH_2Ph$, R_3 = Me] (98) (0.12 g, 22%); mp 145-147°C.

^1H NMR ($CDCl_3$): δ 8.13 (1H, d, J = 8.1 Hz, H-4), 7.38 (1H, t, J = 7.4 Hz, H-6), 7.31-7.20 (6H, m, H-5 and $CH_2\underline{Ph}$), 7.11 (1H, d, J = 7.4 Hz, H-7), 6.60 (1H, br, CONH), 3.75 (2H, br, $COC\underline{H}_2Ph$), 3.64 (3H, s, N-CH_3).

^{13}C NMR ($CDCl_3$): δ 163.42 ($\underline{C}ONH$), 138.37 (s), 128.59, 128.54 (s), 127.63 (s), 127.52, 127.40 (s), 127.20, 126.40 (s), 125.39, 122.52, 122.32, 110.30 (C-7), 42.94 (CH_2Ph), 30.24 (N-CH_3).

Analysis calculated for $C_{34}H_{30}N_4O_2S_2$ requires:
C, 69.1; H, 5.2; N, 9.5; S, 10.8%.
Found: C, 68.6; H, 5.3; N, 9.5; S, 10.6%.

EXAMPLE E

Preparation of Compounds 19 and 83 of Table 1 by the Method of Scheme 4

A mixture of 2-amino-3-methylpyridine (43.28 g, 0.4 mol) and benzotriazole (47.65 g, 0.4 mol) in EtOH (500 mL) was treated over 5 minutes with formaldehyde (32.2 g of 37% solution, 0.4 mol). The mixture was stirred at 20°C overnight, then cooled and filtered to give 2-[(1-benzotriazolyl)methyl]-3-methyl pyridine (30 g, 31%). A sample was crystallized from EtOH; mp 175-177°C.

^1H NMR ($CDCl_3$): δ 8.10 (1H, d, J = 5 Hz, H-8), 8.10 and 8.00 (2H, 2d, J = 8 Hz, H-4',7'), 7.45 and 7.33 (2H, 2t, J = 8 Hz, H-5',6'), 7.25 (1H, d, J = 7 Hz, H-4), 6.54 (1H, dd, J = 7.5 Hz, H-5), 6.47 (2H, d,

J = 7 Hz, CH_2), 5.38 (1H, t, J = 7 Hz, NH), 2.07 (3H, s, CH_3).

Crude 2-[(1-benzotriazolyl)methyl]-3-methyl-pyridine (30 g, 125 mmol) was suspended in dioxan (400 mL) and treated with NaBH4 (5 g, 130 mmol). The mixture was heated under reflux for 8 hours, then the majority of the solvent was removed under reduced pressure. The residue was partitioned between toluene and water, and the organic layer was washed successively with dilute NaOH solution and water, and dried. Removal of the solvent gave 2-methylamino-3-methylpyridine as an oil (12.8 g, 84%).
^{1}H NMR ($CDCl_3$): δ 8.04 (1H, d, J = 5.1 Hz,H, H-6), 7.19 (1H, d, J = 7.1 Hz, H-4), 6.50 (1H, dd, J = 7.1, 5.1 Hz, 5-H), 4.15 (1H, m, NH), 3.03 (3H, d, J = 4.5 Hz, CH_3N), 2.06 (3H, s, CH_3).
^{13}C NMR ($CDCl_3$): δ 157.3 (C-2), 145.0 (C-8), 136.1 (C-4), 116.4 (C-3), 111.9 (C-5), 28.3 (CH_2) and 16.5 (CH_3).

A solution of the above pyridine (6.1 g, 50 mmol) in dry THF (150 mL) was cooled to -78°C under dry N_2, and n-BuLi (19.6 mL of a 2.5 M solution in hexanes, 50 mmol) was added dropwise, followed by t-BuLi (32 mL of a 1.7 M in pentane, 55 mmol). The resulting mixture was allowed to warm to -20°C and maintained at that temperature for 30 minutes before being recooled to -78°C and treated with dry CO_2 gas until the mixture was decolorized. After warming to 20°C, the mixture was acidified with dilute HCl, and the solvent was removed under reduced pressure. The residue was dissolved in EtOH (100 mL) containing p-TsOH (100 mg), heated under reflux for 3 hours to effect ring closure, and neutralized with aqueous ammonia. Solvent was then removed, and the residue was worked up in EtOAc to give

an oil, which was extracted with hot hexane, charcoaled, and filtered through celite. Concentration of the solution and cooled, gave 1-methyl-7-aza-2-indolinone(1,3-dihydro-1-methyl-2H-pyrrolo-(2,3-bipyridin-2-one) [VII: R_1 = 7-aza, R_3 = Me] (1.2 g, 15%); mp (hexane) 94-96°C.

^{1}H NMR ($CDCl_3$): δ 8.15 (1H, d, J = 5.3 Hz, H-8), 7.48 (1H, d, J = 7.2 Hz, H-4), 8.94 (1H, dd, J = 7.2, 5.3 Hz, H-5), 3.53 (2H, s, CH_2), 3.29 (3H, s, CH_3).

^{13}C NMR ($CDCl_3$): δ 174.1 (C-2), 158.1 (C-7a), 146.6 (C-8), 131.3 (C-4), 119.0 (C-3a), 117.8 (C-5), 34.6 (CH_2), 25.1 (CH_3).

P_2S_5 (3.80 g, 8.10 mmol) was added to a vigorously stirred suspension of Na_2CO_3 (0.88 g, 8.10 mmol) in THF (30 mL). After the mixture had become homogeneous (ca. 15 minutes), a solution of 1-methyl-7-aza-2-indolinone [VII: R_1 = 7-aza, R_3 = Me] (1.00 g) in THF (10 mL) was added and stirring was continued for 18 hours at 20°C. Solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and water. Workup of the organic layer, and chromatography of the residue on silica gel (elution with EtOAc/petroleum ether (1:5)) gave 1-methyl-7-aza-2-indolinethione [IX: R_1 = 7-aza, R_3 = Me] (0.81 g, 73%); mp (EtOAc/petroleum ether) 130-133°C.

^{1}H NMR ($CDCl_3$): δ 8.28 (1H, dd, J = 5.2, 0.6 Hz, H-6), 7.57 (1H, dd, J = 7.3, 0.6 Hz, H-4), 7.07 (1H, dd, J = 7.3, 5.2 Hz, H-5), 4.06 (2H, s, H-3), 3.66 (3H, s, N-CH_3).

^{13}C NMR: δ 201.70 (C-2), 159.21 (s), 147.22 (d), 131.39 (d), 123.20 (s), 119.34 (d), 46.98 (C-3), 30.02 (N-CH_3).

Analysis calculated for $C_8H_8N_2S$ requires:
C, 58.5; H, 4.9; N, 17.1; S, 19.5%.
Found: C, 58.3; H, 4.9; N, 17.0; S, 19.8%.

A solution of the above thione (0.70 g, 4.26 mmol) in THF (5 mL) was added dropwise over 5 minutes under N_2 to an ice-cooled suspension of NaH (0.2 g of a 60% w/w dispersion in oil, 6.11 mmol). After gas evolution had ceased (5 minutes), phenyl isocyanate (0.47 mL, 4.25 mmol) was added, and stirring was continued for 1 hour at 20°C. Aqueous 1N HCl was then added, and the mixture was extracted with EtOAc. The organic layer was worked up, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:1) and EtOAc gave foreruns, while elution with EtOAc/MeOH (10:1) gave N-phenyl (1-methyl-7-aza-2-thioxo-3-indolinyl)carboxamide (**19**) [IV: R_1 = 7-aza, R_2 = CONHPh, R_3 = Me] as a fluorescent green solid (0.67 g, 55% yield); mp (after trituration with MeOH) 162-164°C (dec).

^{1}H NMR ($(CD_3)_2SO$): δ 12.46 (1H, s, CONH), 8.68 (1H, dd, J = 7.7, 1.0 Hz, H-6), 8.02 (1H, d, J = 6.0 Hz, H-4), 7.72 (2H, d, J = 8.4 Hz, ArH), 7.36-7.29 (4H, m, ArH), 7.01 (1H, t, J = 7.3 Hz, ArH), 3.80 (3H, s, N-CH_3).

^{13}C NMR: δ 66.96 (C-2), 163.59 (CONH), 140.77 (s), 139.81 (s), 129.29 (d), 128.85 (d), 127.21 (s), 126.84 (d), 122.16 (d), 118.65 (d), 115.92 (d), 48.57 (C-3), 29.18 (N-CH_3).

Analysis calculated for $C_{15}H_{13}N_3O_2S \cdot CH_3OH$ requires:
C, 60.9; H, 5.4; N, 13.3; S, 10.2%.
Found: C, 60.6; H, 5.4; N, 13.4; S, 10.3%.

A solution of sodium perborate (0.50 g, 5.00 mmol) in water (25 mL) was added to a vigorously stirred suspension of the above 7-aza compound (**19**) (0.50 g,

176 mmol) in glacial AcOH (50 mL). After 1 hour the solid was filtered off, washed sequentially with water and Et_2O, and dried to give 2,2'-dithiobis[*N*-phenyl-1-methyl-7-azaindolyl-3-carboxamide] [V: R_1 = 7-aza, R_2 = CONHPh, R_3 = Me] (**83**) (100%); mp 197-198°C. ^1H NMR ($(CD_3)_2SO$): δ 9.49 (1H, s, CONH), 8.36 (1H, dd, J = 4.5, 1.5 Hz, H-6), 8.14 (1H, dd, J = 7.9, 1.5 Hz, H-4), 7.19 (1H, dd, J = 7.9, 4.5 Hz, H-5), 7.16-7.09 (4H, m, ArH), 6.98 (1H, m, ArH), 3.75 (3H, s, N-CH_3). ^{13}C NMR: δ 160.42 (CONH), 147.58 (s), 145.99 (d), 138.29 (s), 129.86 (s), 129.62 (d), 128.25 (d), 123.05 (d), 119.23 (d), 118.09 (s), 117.76 (d), 117.57 (s), 28.61 (N-CH_3).

Analysis calculated for $C_{30}H_{24}N_6O_2S_2 \cdot 2.5H_2O$ requires:
C, 59.1; H, 4.8; N, 13.8; S, 10.5%.
Found: C, 59.1; H, 4.2; N, 13.8; S, 10.5%.

EXAMPLE F

Preparation of Compound 99 of Table 1 by the Method Outlined in Scheme 5

A solution of 2-[(4-methylphenylsulfonyl)methyl]-aniline [XII: R_1 = H, R_2 = Me, X = 4-methylphenyl] (Le Corre M, Hercouet A, Le Stanc Y, Le Baron H, Tetrahedron 1985;22:5313) in dry THF (60 mL), under N_2, was cooled to -78°C and n-butyllithium (9.6 mL, 2.5 M solution in hexanes) was added dropwise. The mixture was allowed to warm to -10°C to give a deep red colored solution which was recooled to -78°C after 30 minutes. CS_2 (3 mL, 5 mmol) was added rapidly, and the mixture was allowed to warm slowly to 20°C. The solvent was removed under vacuum and the residue was diluted with water, and acidified with 2 M HCl. After stirring at 20°C for 3 hours, the solution was extracted with EtOAc and dried (Na_2SO_4). The solvent was removed, and

chromatography of the residue on SiO_2 (CH_2Cl/EtOAc, 9:1) gave bis[3-(4-methylphenylsulfonyl)-2-indolyl]-disulfide [XIII: R_1 = H, R_2 = Me, X = 4-methylphenyl] (99) (0.2 g, 7% yield); mp (benzene) 230-233°C.

^1H NMR ($CDCl_3$): δ 8.06 (1H, m, NH), 7.91 (3H, m, H-4, H-2, and H-4'), 7.45 (1H, m, H-6), 7.21 (4H, m, H-5, H-7, H-3', and H-5'), 2.33 (3H, s, CH_3).

^{13}C NMR ($CDCl_3$): δ 144.1, 140.0, 136.6, 134.0, 129.9 (CH), 126.4 (CH), 125.4, 124.5 (CH), 122.8 (CH), 119.1 (CH), 115.1, 112.2 (CH), and 21.5 (CH_3).

Analysis calculated for $C_{30}H_{24}N_2O_4S_4 \cdot 0.2(C_6H_6)$ requires: C, 60.4; H, 4.1; N, 5.5; S, 20.7%.

Found: C, 60.7; H, 4.4; N, 4.9; S, 21.1%.

EXAMPLE G

Preparation of Compounds 24 and 100 of Table 1 by the Method Outlined in Scheme 6

A stirred solution of benzoyl chloride (from benzoic acid, 0.45 g, 3.68 mmol) in Me_2CO (20 mL) was treated dropwise at 0°C with a solution of NaN_3 (0.26 g, 3.98 mmol) in water (2 mL). After 15 minutes the solution was partitioned between water and benzene, and the organic layer was washed well with $NaHCO_3$ and worked up to give crude phenacyl azide, which was used directly.

A solution of 1-methyl-2-indolinethione (0.50 g, 3.06 mmol) in dry THF (3 mL) was added dropwise at 20°C under N_2 to a stirred suspension of NaH (0.13 g of a 60% w/w suspension in mineral oil, 3.37 mmol) in THF (2 mL). After gas evolution had ceased (5 minutes), a solution of the above phenacyl azide in THF (2 mL) was added dropwise, and the mixture was stirred at 20°C for 1 hour, then poured into 6N HCl and extracted with EtOAc. The residue from the organic layer was

chromatographed on silica gel. Elution with CH_2Cl_2/petroleum ether (3:7) gave foreruns, and elution with CH_2Cl_2/petroleum ether (2:3) gave 3-benzoyl-1-methyl-2-indolinethione [XV: R_1 = H, R_3 = Me, R_5 = C6H5] (**24**) (0.31 g, 38%); mp (trituration from MeOH) 132-134°C.

^{1}H NMR ($CDCl_3$): δ 15.83 (1H, s, SH), 7.68-7.53 (5H, m, COPh), 7.21 (1H, dd, J = 8.1,7.3 Hz, H-5), 7.11 (1H, d, J = 8.1 Hz, H-4), 6.90 (1H, dd, J = 8.0, 7.3 Hz, H-6), 6.76 (1H, d, J = 8.0 Hz, H-7), 3.74 (3H, s, NCH_3).

^{13}C NMR ($CDCl_3$): δ 181.71 (COPh), 175.09 (C-2), 141.42 (s), 134.87 (s), 131.29, 128.85, 128.37, 125.64 (4xd), 125.22 (s), 122.81, 120.31 (2xd), 111.77 (s), 109.129 (d), 29.57 (NCH_3).

Analysis calculated for $C_{16}H_{13}NOS$ requires:
C, 71.9; H, 4.9; N, 5.2; S, 12.0%.
Found: C; 71.6; H, 5.1; N, 6.2; S, 13.9%.

A solution of **24** (0.10 g, 0.37 mmol) in CH_2Cl_2 (20 mL) was treated dropwise at 20°C with a solution of I_2 (0.50 g) in CH_2Cl_2 (5 mL), until TLC indicated complete conversion (ca. 2 hours). The solution was concentrated to ca. 1 mL and chromatographed directly on silica gel. Elution with CH_2Cl_2 gave traces of I_2 and starting material, and further elution with CH_2Cl_2/MeOH (19:1) gave bis[3-benzoyl-1-methylindole-(2)]disulfide [XVI: R_1 = H, R_3 = Me, R_5 = C_6H_5] (**100**) (0.06 g, 61%); mp ($CHCl_3$/petroleum ether) 199-202°C.

^{1}H NMR (CD_3SOCD_3): δ 7.56 (1H, d, J = 8.4 Hz, H-4), 7.50 (1H, d, J = 8.1 Hz, H-7), 7.46 (dd, J = 8.1, 7.4 Hz, H-6), 7.35 (1H, dd, J = 8.4, 7.4 Hz, H-5), 7.19 (3H, m, H-2',4',6'), 6.92 (2H, d, J = 7.1 Hz, H-3',5'), 3.48 (3H, s, NCH_3).

^{13}C NMR (CD_3SOCD_3): δ 190.20 (COPh), 140.05, 138.03, 132.75 (3xs), 131.60, 128.48, 127.88 (3xd), 126.00 (s),

124,78, 122.27 (2xd), 122.03 (s), 121.03, 111.20 (2xd), 30.37 (NCH_3).

Analysis calculated for $C_{32}H_{24}N_2O_2S_2$ requires:
C, 69.8; H, 4.8; N, 5.1; S, 11.6%.
Found: C, 70.3; H, 4.7; N, 5.2; S, 11.3%.

Compounds 25, 26, 101, and 102 of Table 1

Similar treatment of 1-methyl-2-indolinethione with 4-carbomethoxybenzoyl azide gave 3-(4'-carbomethoxybenzoyl)-1-methyl-2-indolinethione [XV: R_1 = H, R_3 = Me, R_5 = 4-$MeOOCC_6H_4$] (**26**) (68%); mp 164-166°C.
^1H NMR ($CDCl_3$): δ 15.85 (1H, s, SH), 8.23 (2H, d, J = 8.3 Hz, H-3',5'), 7.76 (2H, d, J = 8.3 Hz, H-2',6'), 7.23 (1H, dd, J = 8.0, 7.6 Hz, H-5'), 7.12 (1H, d, J = 7.6 Hz, H-4), 6.90 (1H, dd, J = 8.0, 7.9 Hz, H-6), 6.69 (1H, d, J = 7.9 Hz, H-7), 3.99 (3H, s, $COOCH_3$), 3.74 (3H, s, NCH_3).
^{13}C NMR ($CDCl_3$): δ 182.07 ($\underline{C}OAr$), 173.27 (C-2), 166.31 ($\underline{C}OOCH_3$), 141.59, 138.92, 132.51 (3xs), 130.11, 128.54, 126.04 (3xd), 124.76 (s), 123.00, 120.26 (2xd), 119.95 (s), 109.28 (d), 52.50 ($COO\underline{C}H_3$), 29.61 (NCH_3).
Analysis calculated for $C_{18}H_{15}NO_3S$ requires:
C, 66.4; H, 4.7; N, 4.3; S, 9.8%.
Found: C, 66.5; H, 4.7; N, 4.6; S, 9.8%.

Oxidation of **26** with I_2/CH_2Cl_2 as above gave bis[3-(4'-carbomethoxybenzoyl)-1-methylindole-(2)]-disulfide [XVI: R_1 = H, R_3 = Me, R_5 = 4-$MeOOCC_6H_4$] (**102**); mp ($CHCl_3$/petroleum ether) 200-203°C.
^1H NMR (CD_3SOCD_3): δ 7.74 (2H, d, J = 8.4 Hz, H-3',5'), 7.67 (1H, d, J = 8.0 Hz, H-4), 7.64 (1H, d, J = 8.4 Hz, H-7), 7.44 (1H, dd, J = 8.4, 8.0 Hz, H-6), 7.27 (1H, dd, J = 8.0, 8.0 Hz, H-5), 6.99 (2H, d, J = 8.4 Hz, H-2',6'), 3.91 (3H, s, $COOCH_3$), 3.51 (3H, s, NCH_3).

^{13}C NMR (CD_3SOCD_3): δ 189.31 (COAr), 165.56 ($COOCH_3$), 143.77, 137.98, 133.31, 131.61 (4xs), 128.50, 128.33 (2xd), 125.87 (s), 124.99, 122.62 (2xd), 121.27 (s), 121.09, 111.22 (2xd), 52.34 ($COOCH_3$), 30.33 (NCH_3).

Analysis calculated for $C_{36}H_{28}N_2O_6S_2$ requires:

C, 66.6; H, 4.4; N, 4.3; S, 9.9%.

Found: C, 66.2; H, 4.8; N, 4.4; S, 9.9%.

A suspension of **26** (0.1 g, 0.31 mmol) in MeOH (5 mL) containing 3N NaOH (2 mL) was stirred at 20°C for 3 hours, then concentrated to dryness. The residue was dissolved in water and acidified (concentrated HCl) to give 3-(4'-carboxybenzoyl)-1-methyl-2-indolinethione [XV: R_1 = H, R_3 = Me, R_5 = 4-$HOOCC_6H_4$] (**25**) (100%); mp 282°C (dec).

^1H NMR (CD_3SOCD_3/CD_3COCD_3): δ 15.90 (0.3H, br, SH), 13.00 (1H, br s, COOH), 8.26 (2H, d, J = 8.2 Hz, H-3',5'), 8.10 (0.6H, s, SH), 7.85 (2H, d, J = 8.2 Hz, H-2',6'), 7.40 (1H, d, J = 8.0 Hz, H-4), 7.29 (1H, dd, J = 8.0, 8.0 Hz, H-5), 6.98 (1H, dd, J = 8.0, 7.5 Hz, H-6), 6.68 (1H, d, J = 7.5 Hz, H-7), 3.77 (3H, s, NCH_3).

^{13}C NMR CD_3SOCD_3/CD_3COCD_3): δ 167.57, 167.50 (COAr and COOH), 142.40, 135.64, 134.55 (3xs), 130.86, 130.18, 129.13, 126.93 (4xd), 125.17 (s), 123.81, 120.68 (2xd), 112.39 (s), 110.82 (d), 29.94 (NCH_3).

Analysis calculated for $C_{17}H_{13}NSO_3 \cdot H_2O$ requires:

C, 64.6; H, 4.3; N, 4.4; S, 10.1%.

Found: C, 64.6; H, 4.4; N, 4.0; S, 9.6%.

Similar hydrolysis of **102** gave bis[3-(4'-carboxybenzoyl)-1-methylindole-(2)]disulfide [XVI: R_1 = H, R_3 = Me, R_5 = 4-$HOOCC_6H_4$] (**101**); mp ($CHCl_3$/petroleum ether) 241-246°C.

^1H NMR (CD_3SOCD_3): δ 12.62 (1H, br, COOH), 7.89 (3H, m, H-4 and H-3',5'), 7.74 (1H, d, J = 8.5 Hz, H-7),

7.58 (3H, m, H-6 and H-2',6'), 7.36 (1H, m, H-5), 3.66 (3H, s, NCH_3).

Analysis calculated for $C_{34}H_{24}N_2O_6S_2 \cdot 0.5 \cdot H_2O$ requires:
C, 63.1; H, 4.2%.
Found: C, 63.1; H, 5.3%.

EXAMPLE H

Preparation of Compounds 104 and 105 of Table 1 by the Method Outlined in Scheme 7

A solution of monomethyl terephthalate [XVII: 4-COOMe] (1.32 g, 7.33 mmol) and DMF (1 drop) in $SOCl_2$ (30 mL) was heated under reflux for 45 minutes, before concentration to dryness under reduced pressure. The residue was dissolved in benzene and evaporated to dryness again. The crude acid chloride was dissolved in dry Me_2CO (20 mL), cooled to 0°C, and treated with a solution of NaN_3 (0.52 g, 8.00 mmol) in water (3 mL). After 20 minutes the solution was diluted with water, extracted with CH_2Cl_2, and worked up to give the crude acyl azide [XVIII: 4-COOMe], which was immediately dissolved in dry toluene (25 mL) and heated under reflux under N_2 for 2 hours. Concentration to dryness under reduced pressure afforded the isocyanate [XIX: 4-COOMe] which was used directly.

A solution of 1-methyl-2-indolinethione [IV: R_1,R_2 = H, R_3 = CH_3] (1.00 g, 6.13 mmol) in THF (2 mL) was added under N_2 to a suspension of NaH (0.26 g of 60% w/w dispersion in mineral oil, 6.50 mmol) in THF (15 mL). After gas evolution had ceased (5 minutes), a solution of the above crude isocyanate in THF (10 mL) was added, and the solution was stirred at 20°C for a further 1 hour. The mixture was acidified with 3N HCl, extracted with EtOAc and

worked up to give an oily solid. Chromatography on silica gel, eluting with EtOAc, afforded a greenish solid. This was dissolved in MeOH and treated with 30% H_2O_2 (0.20 mL), and the resulting yellow precipitate was filtered off and washed well with MeOH to give 2,2'-dithiobis[*N*-(4'-carbomethoxy)phenyl-1-methylindolyl-3-carboxamide] (**104**) [XX: R = 4-COOMe] (0.74 g, 35%); mp 184-186°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.87 (1H, br, CONH), 7.80 (1H, d, J = 8.0 Hz, H-4), 7.74 (2H, d, J = 8.7 Hz, H-2',6'), 7.37 (1H, d, J = 8.3 Hz, H-7), 7.32 (2H, d, J = 8.7 Hz, H-3',5'), 7.26 (1H, dd, J = 8.3, 7.6 Hz, H-6), 7.15 (1H, dd, J = 8.0, 7.6 Hz, H-5), 3.84 (3H, s, CO_2CH_3), 3.66 (3H, s, N-CH_3).

^{13}C NMR: δ 165.79 ($\underline{C}OOCH_3$), 161.56 (CONH), 143.01 (s), 137.68 (s), 129.79 (d), 125.41 (s), 124.35 (d), 123.37 (s), 121.40 (d), 120.82 (d), 119.90 (s), 118.33 (d), 117.93 (s), 110.74 (d), 51.74 ($COO\underline{C}H_3$), 30.04 (N-CH_3).

Analysis calculated for $C_{36}H_{30}N_4O_6S_2 \cdot H_2O$ requires:
C, 62.1; H, 4.6; N, 8,1; S, 9.2%.
Found: C, 62.2; H, 4.6; N, 8.0; S, 9.2%.

A suspension of (**104**) (0.23 g, 0.34 mmol) in MeOH (40 mL) was treated with 3N KOH (15 mL) and stirred at 20°C for 90 minutes. The resulting solution was filtered, acidified, and the resulting precipitate collected and re-dissolved in CH_2Cl_2 (10 mL) containing MeOH (1 mL). H_2O_2 (0.20 mL of 30%) was added, and after 1 hour the solvents were removed under reduced pressure. The residue was triturated with MeOH to give 2,2'-dithiobis[*N*-(4'-carboxy)phenyl-1-methylindolyl-3-carboxamide] (**105**) [XX: R = 4-COOH] (100% yield); mp 221°C (dec).

^{1}H NMR ($(CD_3)_2SO$): δ 12.63 (1H; br, COOH), 9.78 (1H, s, CONH), 7.80 (1H, d, J = 8.0 Hz, H-4), 7.72 (2H, d,

J = 8.7 Hz, H-3',5'), 7.39 (1H, d, J = 8.4 Hz, H-7), 7.30 (2H, d, J = 8.7 Hz, H-2',6'), 7.28 (t, J = 8.4, 7.7 Hz, H-6), 7.16 (1H, t, J = 8.0, 7.7 Hz, H-5), 3.66 (3H, s, N-CH_3).

^{13}C NMR: δ 166.95 (COOH), 161.58 (CONH), 142.67 (s), 137.78 (s), 129.99 (d), 129.81 (s), 125.41 (s), 124.72 (s), 124.54 (d), 121.50 (d), 120.93 (d), 118.39 (d), 110.89 (d), 30.12 (N-CH_3).

Analysis calculated for $C_{34}H_{26}N_4O_6S_2 \cdot 0.5H_2O$ requires:

C, 61.9; H, 4.1; N, 8.5; S, 9.7%.

Found: C, 61.6; H, 4.2; N, 8.4; S, 9.9%.

Compounds 106 and 107 of Table 1

Similar treatment of 1-methyl-2-indolinethione [IV: R_1,R_2 = H, R_3 = CH_3] with the isocyanate [XIX: 3-COOMe] derived from monomethyl isophthalate gave 2,2'-dithiobis[*N*-(3'-carbomethoxy)phenyl-1-methylindolyl-3-carboxamide] (**106**) [XX: R = 3-COOMe] (57% yield); mp 193-195°C.

^{1}H NMR ($(CD_3)_2SO$): δ 9.67 (1H, s, CONH), 7.96 (1H, br s, H-2'), 7.79 (1H, d, J = 8.0 Hz, H-4), 7.56 (1H, d, J = 7.7 Hz, H-6'), 7.45 (1H, d, J = 8.2 Hz, H-7), 7.34 (1H, d, J = 8.3 Hz, H-4'), 7.28 (1H, dd, J = 8.3, 7.7 Hz, H-5'), 7.21 (1H, dd, J = 8.2, 7.7 Hz, H-6), 7.10 (1H, dd, J = 8.0, 7.7 Hz, H-5), 3.88 (3H, s, $COOCH_3$), 3.66 (3H, s, N-CH_3).

^{13}C NMR: δ 166.04 ($\underline{C}OOCH_3$), 161.48 (CONH), 138.89 (s), 137.63 (s), 129.77 (s), 129.54 (s), 128.62 (d), 125.21 (s), 124.39 (d), 123.51 (s), 121.28 (d), 120.83 (d), 119.50 (d), 118.31 (s), 110.64 (d), 51.99 (COO$\underline{C}H_3$), 30.02 (N-CH_3).

Analysis calculated for $C_{36}H_{30}N_4O_6S_2$ requires:

C, 63.7; H, 4.5; N, 8.3; S, 9.5%.

Found: C, 63.9; H, 4.6; N, 8.4; S, 9.6%.

Hydrolysis of the ester (**106**) as above, followed by re-oxidation with H_2O_2/MeOH, gave 2,2'-dithiobis[*N*-(3-carboxy)phenyl-1-methylindolyl-3-carboxamide] (**107**) [XX: R = 3-COOH] (97% yield); mp 219-220°C.

^{1}H NMR ($(CD_3)_2SO$): δ 12.68 (1H, br, COOH), 9.69 (1H, s, CONH), 7.98 (1H, br s, H-2'), 7.80 (1H, d, J = 8.0 Hz, H-4), 7.56 (1H, d, J = 7.7 Hz, H-6'), 7.43 (1H, d, J = 8.2 Hz, H-7), 7.36 (1H, d, J = 8.3, 7.7 Hz, H-4'), 7.24 (2H, m, H-5',6), 7.11 (1H, t, J = 8.0, 7.7 Hz, H-5), 3.66 (3H, s, N-CH_3).

^{13}C NMR: δ 167.10 (COOH), 161.53 (CONH), 138.77 (s), 137.62 (s), 130.92 (s), 129.47 (s), 128.44 (d), 125.18 (s), 124.45 (d), 123.75 (d), 123.31 (d), 121.32 (d), 120.81 (d), 119.91 (d), 118.51 (s), 110.67 (d), 30.01 (N-CH_3).

Analysis calculated for $C_{34}H_{26}N_4O_6S_2 \cdot 0.5H_2O$ requires:
C, 61.9; H, 4.1; N, 8.5; S, 9.7%.
Found: C, 61.7; H, 4.3; N, 8.8; S, 9.7%.

Compounds **108** & **109** of Table 1

Similar treatment of 1-methyl-2-indolinethione [IV: R_1,R_2 = H, R_3 = CH_3] with the isocyanate [XIX: 2-COOMe] derived from monomethyl phthalate gave 2,2'-dithiobis[*N*-(2-carbomethoxy)phenyl-1-methyl-indolyl-3-carboxamide] (**108**) [XX: R = 2-COOMe] (61% yield); mp 179-181°C.

^{1}H NMR ($(CD_3)_2SO$): δ 10.82 (1H, s, CONH), 7.89 (2H, 2xd, J = 8.3, 8.0 Hz, H-3',6'), 7.74 (1H, d, J = 8.3 Hz, H-4), 7.32 (2H, m, H-7,4'), 7.20 (1H, dd, J = 8.1, 7.5 Hz, H-6), 7.12 (1H, dd, J = 8.3, 7.5 Hz, H-5), 6.97 (1H, m, H-5'), 3.84 (3H, s, $COOCH_3$), 3.68 (3H, s, N-CH_3).

Analysis calculated for $C_{36}H_{30}N_4O_6S_2 \cdot 0.5H_2O$ requires:
C, 62.9; H, 4.5; N, 8.2; S, 9.3%.
Found: C, 62.8; H, 4.5; N, 8.1; S, 9.3%.

Hydrolysis of the ester (**108**) as above, followed by re-oxidation with H_2O_2/MeOH, gave 2,2'-dithiobis[*N*-(2'-carboxy)phenyl-1-methylindolyl-3-carboxamide] (**109**) [XX: R = 2-COOH] (91% yield); mp 184-186°C.
^{1}H NMR ($(CD_3)_2SO$): δ 13.33 (1H, br, COOH), 11.31 (1H, s, CONH), 7.95 (1H, d, J = 8.1 Hz, H-6'), 7.90 (1H, d, J = 7.9 Hz, H-3'), 7.83 (1H, d, J = 8.3 Hz, H-4), 7.30 (2H, m, H-7,4'), 7.19 (1H, dd, J = 8.0, 7.5 Hz, H-6), 7.08 (1H, dd, J = 8.3, 7.5 Hz, H-5), 7.02 (1H, dd, J = 8.1, 7.8 Hz, H-5'), 3.67 (3H, s, N-CH_3).
^{13}C NMR: δ 169.16 (COOH), 160.71 (CONH), 140.55 (s), 137.78 (s), 133.31 (d), 130.50 (d), 129.30 (s), 125.01 (s), 124.50 (d), 121.79 (d), 121.47 (d), 121.05 (d), 120.28 (d), 118.21 (s), 115.91 (s), 110.68 (d), 29.93 (N-CH_3).
Analysis calculated for $C_{34}H_{26}N_4O_6S_2 \cdot 2H_2O$ requires:
C, 59.5; H, 4.4; N, 8.2; S, 9.3%.
Found: C, 59.3; H, 4.3; N, 8.3; S, 9.6%.

Compound **110** of Table 1

Similar treatment of 1-methyl-2-indolinethione [IV: R_1,R_2 = H, R_3 = CH_3] with the isocyanate derived from 4-(carbomethoxy)phenylacetic acid gave 2,2'-dithiobis[*N*-(4'-carbomethoxy)benzyl 1-methylindolyl-3-carboxamide] (**110**) [V: R_1 = H, R_2 = $CONHCH_2Ph\{4\text{-}COOMe\}$, R_3 = Me] (38% yield); mp 178-180°C.
^{1}H NMR ($(CD_3)_2SO$): δ 8.18 (1H, br, CONH), 7.88 (1H, d, J = 8.1 Hz, H-4), 7.82 (2H, d, J = 7.9 Hz, C-2',6'), 7.55 (1H, d, J = 8.3 Hz, H-7), 7.35 (1H, dd, J = 8.3, 7.7 Hz, H-6), 7.28 (2H, d, J = 7.9 Hz, C-3',5'), 7.20

(1H, dd, J = 8.1, 7.7 Hz, H-5), 4.06 (2H, d, J = 5.1 Hz, CONHCH_2), 3.83 (3H, s, COOCH_3), 3.61 (3H, s, N-CH_3).

^{13}C NMR: δ 165.98 (C=OOCH$_3$), 163.17 (CONH), 145.10 (s), 137.61 (s), 129.06 (d), 129.00 (s), 127.85 (s), 126.95 (d), 125.37 (s), 124.31 (d), 121.22 (d), 121.09 (d), 117.89 (s), 110.78 (d), 51.89 (COOCH$_3$), 41.90 (CH_2Ar), 29.94 (N-CH_3).

Analysis calculated for $C_{38}H_{34}N_4O_6S_2 \cdot 0.5H_2O$ requires:
C, 63.8; H, 4.9; N, 7.8; S, 8.9%.
Found: C, 63.7; H, 4.8; N, 7.8; S, 9.1%.

EXAMPLE I

Preparation of Compound 111 of Table 1 by the Method Outlined in Scheme 8.

A solution of 2-chloro-1-methylindole-3-carboxylic acid [XXI] (Marchetti L, Andreani A, Ann. Chim. (Rome) 1973;63:681) (0.95 g, 4.52 mmol) and $SOCl_2$ (0.99 mL, 13 mmol) in 1,2-dichloroethane (100 mL) containing DMF (1 drop) was heated under reflux under N_2 for 2 hours, then concentrated to dryness. The residue was dissolved in CH_2Cl_2 (50 mL) and treated with a slurry of methyl 4-(aminomethyl)benzoate hydrochloride (Nair MG, Baugh CM, J. Org. Chem. 1973;38:2185) (1.00 g, 4.98 mmol) and Et_3N (1.58 mL, 11 mmol) in CH_2Cl_2 (50 mL). After vigorous stirring at 20°C for 24 hours, the mixture was washed with water and the organic portion worked up to give *N*-(4'-carbomethoxy)-benzyl 2-chloro-1-methylindole-3-carboxamide [XXII: R_6 = H, R_7 = CH_2Ph{4-COOMe}] (1.40 g, 86%) which crystallized from aqueous acetone; mp 108-110°C. ^{1}H NMR ((CD_3)$_2$SO): δ 8.38 (1H, t, J = 5.8 Hz, CONHCH_2), 7.95 (2H, d, J = 7.9 Hz, H-2',6'), 7.91 (1H, d, J = 7.8 Hz, H-4), 7.56 (1H, d, J = 7.9 Hz, H-7), 7.52

(2H, d, J = 7.9 Hz, H-3',5'), 7.29 (1H, dd, J = 7.9, 7.1 Hz, H-6), 7.19 (1H, dd, J = 7.8, 7.1 Hz, H-5), 4.60 (2H, d, J = 5.8 Hz, CONHC$\underline{H}_2$), 3.84 (3H, s, COOCH_3), 3.79 (3H, s, N-CH_3).

^{13}C NMR: δ 166.09 (C$\underline{O}$OCH$_3$), 162.77 (CONH), 145.65 (s), 135.00 (s), 129.18 (d), 129.14 (d), 127.94 (s), 127.34 (d), 127.25 (d), 126.34 (s), 124.77 (s), 122.57 (d), 121.19 (d), 119.97 (d), 110.21 (s), 107.11 (d), 51.95 (COO$\underline{C}$H$_3$), 42.15 (CH_2), 29.97 (N-CH_3).

Analysis calculated for $C_{19}H_{17}ClN_2O_3$ requires:
C, 64.0; H, 4.8; N, 7.9; Cl, 9.9%.
Found: C, 64.0; H, 4.8; N, 7.6; Cl, 9.8%.

A solution of the above carboxamide (1.00 g, 2.80 mmol) in DMA (10 mL) was added under N_2 to a stirred suspension of MeSLi (1.06 g, 19 mmol) in DMA (25 mL). After warming at 80°C for 6 hours, the mixture was acidified with 3N HCl, extracted with CH_2Cl_2, and worked up to give a yellow oil. Traces of DMA were removed under high vacuum, and the residue was dissolved in MeOH (20 mL) and treated dropwise with H_2O_2 (0.60 mL of 30% solution). After chilling at -30°C overnight, the precipitate was filtered off, washed well with MeOH, and dried to give 2,2'-dithiobis[*N*-(4'-carboxy)benzyl 1-methylindol-3-carboxamide] (**111**) [V: R_1 = H, R_2 = CONHCH_2Ph{4-COOH}, R_3 = Me] (0.68 g, 72%); mp 178-180°C.

^{1}H NMR ((CD_3)$_2$SO): δ 12.86 (1H, br, COOH), 8.13 (1H, t, J = 5.8 Hz, CON$\underline{H}$CH_2), 7.92-7.80 (3H, m, H-4,2',6'), 7.56 (1H, d, J = 8.3 Hz, H-7), 7.37 (1H, t, J = 8.3, 7.8 Hz, H-6), 7.27 (2H, d, J = 8.3 Hz, H-3',5'), 7.20 (1H, dd, J = 8.1, 7.8 Hz, H-5), 4.02 (2H, d, J = 5.8 Hz, CONHC$\underline{H}_2$), 3.62 (3H, s, N-CH_3).

^{13}C NMR: δ 167.08 (COOH), 163.08 (CONH), 144.51 (s), 137.64 (s), 130.35 (s), 129.25 (d), 129.04 (s), 126.85 (d), 125.25 (s), 124.44 (d), 121.23 (d), 121.10 (d), 118.33 (s), 110.87 (d), 41.92 (CH_2), 29.94 (N-CH_3).

Analysis calculated for $C_{36}H_{30}N_4O_6S2 \cdot 1.5H_2O$ requires:

C, 61.3; H, 4.7; N, 7.9; S, 9.1%.

Found: C, 61.1; H, 4.8; N, 8.3; S, 9.0%.

Compound 112 of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXI] with $SOCl_2$ and glycine methyl ester hydrochloride gave *N*-carbomethoxymethyl 2-chloro-1-methylindole-3-carboxamide [XXII: R_6 = H, R_7 = CH_2COOMe] (78% yield); mp ($CHCl_3$/light petroleum) 102.5-104°C.

^1H NMR ($CDCl_3$): δ 8.26 (1H, d, *J* = 8.1 Hz, H-4), 7.30-7.23 (3H, m, H-5,6,7), 6.96 (1H, br, CONH), 4.32 (2H, d, *J* = 5.0 Hz, $C\underline{H}_2NHCO$), 3.81 (3H, s, $COOCH_3$), 3.75 (3H, s, N-CH_3).

^{13}C NMR: δ 170.91 ($\underline{C}OOCH_3$), 163.48 (CONH), 135.45 (s), 126.90 (s), 125.93 (s), 123.24 (d), 122.25 (d), 121.30 (d), 109.26 (d), 106.32 (s), 52.41 ($COO\underline{C}H_3$), 41.38 ($C\underline{H}_2COOMe$), 30.11 (N-CH_3).

Analysis calculated for $C_{13}H_{13}ClN_2O_3$ requires:

C, 55.6; H, 4.7; N, 10.0%.

Found: C, 55.3; H, 4.8; N, 10.2%.

Treatment of this with MeSLi as above gave 2,2'-dithiobis[*N*-carboxymethyl 1-methylindolyl-3-carboxamide] (112) [V: R_1 = H, R_2 = $CONHCH_2COOH$, R_3 = Me] (56% yield); mp 197°C (dec).

^1H NMR ($(CD_3)_2SO$): δ 7.98 (1H, d, *J* = 8.1 Hz, H-4), 7.59 (1H, br, CONH), 7.55 (1H, d, *J* = 8.4 Hz, H-7), 7.35 (1H, dd, *J* = 8.4, 7.5 Hz, H-6), 7.20 (1H, dd,

J = 8.1, 7.5 Hz, H-5), 3.68 (3H, s, N-CH_3), 3.20 (2H, d, J = 5.2 Hz, $C\underline{H}_2COOH$).

^{13}C NMR: δ 171.02 (COOH), 162.57 (CONH), 137.60 (s), 125.36 (s), 124.30 (d), 121.27 (d), 121.11 (d), 117.69 (s), 110.65 (d), 40.35 (CH_2), 29.87 (N-CH_3).

Analysis calculated for $C_{24}H_{22}N_4O_6S_2 \cdot H_2O$ requires:
C, 52.9; H, 4.4; N, 10.3; S, 11.8%.
Found: C, 52.5; H, 4.5; N, 10.0; S, 11.2%.

Compound 113 of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXI] with $SOCl_2$ and N-methylaniline gave *N*-methyl-*N*-phenyl 2-chloro-1-methylindole-3-carboxamide [XXII: R_6 = Me; R_7 = Ph] (67% yield); mp (Me_2CO/water) 163°C.

^1H NMR ($(CD_3)_2SO$): δ 7.47 (1H, d, J = 7.6 Hz, H-4), 7.41 (1H, d, J = 8.3 Hz, H-7), 7.22-7.00 (7H, m, ArH), 3.63 (3H, s, N-CH_3), 3.42 (3H, s, N-CH_3).

^{13}C NMR: δ 164. 33 (CONMePh), 143.88 (s), 134.69 (s), 128.50 (d), 125.90 (d), 125.70 (d), 124.86 (s), 124.21 (s), 122.24 (d), 120.71 (d), 118.94 (d), 110.06 (d), 108.80(s), 37.40 (N-CH_3), 29.77 (N-CH_3).

Analysis calculated for $C_{17}H_{15}ClN_2O$ requires:
C, 68.3; H, 5.1; N, 9.4; Cl, 11.9%.
Found: C, 68.4; H, 5.1; N, 9.3; Cl, 12.1%.

Treatment of this with MeSLi as above gave 2,2'-dithiobis[*N*-methyl-*N*-phenyl-1-methylindolyl-3-carboxamide] (113) [V: R_1 = H, R_2 = CON(Me)Ph, R_3 = Me] (53% yield), mp 158-163°C.

^1H NMR ($(CD_3)_2SO$): δ 7.80 (1H, d, J = 7.5 Hz, H-4), 7.57 (1H, d, J = 7.8 Hz, H-7), 7.33-6.99 (7H, m, ArH), 3.86 (3H, s, N-CH_3), 3.33 (3H, s, N-CH_3).

^{13}C NMR: δ 164.14 (CONMePh), 137.59 (s), 129.94 (s), 124.21 (s), 123.73 (s), 123.24 (d), 122.34 (d), 120.25

(d), 119.56 (d), 118.79 (d), 115.43 (s), 110.27 (d), 39.68 (N-CH_3), 30.99 (N-CH_3).

Analysis calculated for $C_{34}H_{31}N_4S_2O_2$ requires:

$[M + H]^+$ 591.3447.

Found: $[M + H]^+$ 591.3441 (FAB mass spectrum).

Analysis calculated for $C_{34}H_{30}N_4S_2O_2$ requires:

C, 69.1; H, 5.1; N, 9.5; S, 10.9%.

Found: C, 69.2; H, 5.2; N, 9.6; S, 10.6%.

Compound 114 of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXI] with $SOCl_2$ and 3-aminopropane-1,2-diol gave *N*-(2,3-dihydroxypropyl)-2-chloro-1-methylindole-3-carboxamide [XXII: R_6 = H;, R_7 = $CH_2CH(OH)CH_2OH$] (46%) as an oil.

^1H NMR ($(CD_3)_2SO/D_2O$): δ 7.94 (1H, d, J = 7.0 Hz, H-4), 7.53 (1H, d, J = 7.2 Hz, H-7), 7.38-7.19 (2H, m, H-5,6), 3.78 (3H, s, N-CH_3), 3.68-3.26 (5H, m, $CH_2CHOHCH_2OH$).

^{13}C NMR: δ 162.72 (CONH), 134.94 (s), 125.94 (s), 124.79 (s), 122.52 (d), 121.15 (d), 120.05 (d), 110.17 (d), 107.09 (d), 70.17 (CHOH), 63.90 (CH_2OH), 42.34 ($CONHCH_2$), 29.97 (N-CH_3).

Analysis calculated for $C_{13}H_{15}ClN_2O_3$ requires:

M^+ 284.0742, 282.0771.

Found: M^+ 284.0744, 282.0763 (mass spectrum).

Treatment of this with MeSLi as above gave 2,2'-dithiobis[*N*-(2,3-dihydroxypropyl)-1-methyl-indolyl-3-carboxamide] (**114**) [V: R_1 = H, R_2 = $CONHCH_2CH(OH)CH_2OH$, R_3 = Me] (71% yield) as a yellow foam; mp 198°C (dec).

^1H NMR ($(CD_3)_2SO/D_2O$): δ 7.89 (1H, d, J = 8.1 Hz, H-4), 7.56 (1H, d, J = 8.4 Hz, H-7), 7.42 (1H, dd, J = 8.4, 7.3 Hz, H-6), 7.27 (1H, dd, J = 8.1, 7.3 Hz,

H-5), 3.75 (3H, s, N-CH_3), 3.40-3.20 (5H, m, $CH_2CHOHCH_2OH$).

^{13}C NMR: δ 162.61 (CONH), 137.70 (s), 125.21 (s), 124.40 (d), 121.34 (d), 121.27 (d), 120.81 (s), 117.85 (s), 110.88 (d), 70.17 (CHOH), 63.75 (CH_2OH), 41.96 ($CONHCH_2$), 29.95 (N-CH_3).

Analysis calculated for $C_{26}H_{30}N_4O_6S_2$ requires:
C, 55.9; H, 5.4; N, 10.0; S, 11.5%.
Found: C, 55.4; H, 5.4; N, 9.7; S, 11.5%.

Compound 115 of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXI] with $SOCl_2$ and *N,N*-dimethyl-ethylenediamine, followed by extraction into 3N HCl, neutralization with aqueous NH_3 and extraction with EtOAc gave *N,N*-dimethylaminoethyl-2-chloro-1-methylindole-3-carboxamide [XXII: R_6 = H, R_7 = $CH_2CH_2NMe_2$] as an oil (74% yield), which eventually solidified; mp 63°C.

^1H NMR ($CDCl_3$): δ 8.20 (1H, dd, J = 8.1, 1.7 Hz, H-4), 7.26-7.20 (3H, m, H-5,6,7), 7.01 (1H, br, CONH), 3.69 (3H, s, N-CH_3), 3.58 (2H, dt, J = 6.1, 5.1 Hz, $CONHCH_2$), 2.55 (2H, t, J = 6.1 Hz, $CH_2N(CH_3)_2$, 2.30 (6H, s, $N(CH_3)$).

^{13}C NMR: δ 163.62 (CONH), 135.31 (s), 126.43 (s), 125.79 (s), 122.90 (d), 121.83 (d), 121.06 (d), 109.17 (d), 107.07 (s), 57.84 ($CONHCH_2$), 45.14 ($N(CH_3)_2$), 36.80 $CH_2N(CH_3)_2$), 29.96 (N-CH_3).

Analysis calculated for $C_{14}H_{18}ClN_3O$ requires:
M^+ 281.1109, 279.1138.
Found: M^+ 281.1106, 279.1118 (mass spectrum).

Following treatment of this with MeSLi as above, the reaction mixture was partitioned between CH_2Cl_2 and water. The organic portion was extracted with 3N HCl,

and the extract was neutralized with aqueous NH_3, extracted with CH_2Cl_2, and worked up to give an oil which was dissolved in MeOH and allowed to stand at 20°C for 48 hours. The product was adsorbed directly onto silica and chromatographed. Elution with MeOH/EtOAc (1:19) containing a trace of concentrated NH_4OH gave 2,2'-dithiobis[*N*-(*N*,*N*-dimethylaminoethyl) 1-methylindolyl-3-carboxamide] (**115**) [V: R_1 = H, R_2 = $CONHCH_2CH_2NMe_2$, R_3 = Me] (54% yield); mp (CH_2Cl_2/light petroleum) 163.5-165°C.

^1H NMR ($CDCl_3$): δ 8.24 (1H, d, J = 8.1 Hz, H-7), 7.36 (1H, dd, J = 8.2, 7.8 Hz, H-6), 7.30 (1H, d, J = 8.2 Hz, H-7), 7.25 (1H, dd, J = 8.1, 7.8 Hz, H-5), 7.10 (1H, br, CONH), 3.60 (3H, s, N-CH_3), 2.99 (2H, dt, J = 6.3, 5.5 Hz, CONHC$\underline{H}_2$), 2.26 (2H, t, J = 6.3 Hz, C$\underline{H}_2N(CH_3)_2$), 2.21 (6H, s, $N(CH_3)_2$).

^{13}C NMR: δ 163.71 (CONH), 138.27 (s), 126.64 (s), 125.20 (d), 122.70 (d), 122.11 (d), 118.46 (s), 110.08 (d), 57.72 (CONH$\underline{C}H_2$), 45.19 ($N(CH_3)_2$), 36.81 ($\underline{C}H_2N(CH_3)_2$), 30.15 (N-$CH_3$).

Analysis calculated for $C_{28}H_{36}N_6O_2S_2$ requires:
C, 60.8; H, 6.6; N, 15.2; S, 11.6%.
Found: C, 60.7; H, 6.8; N, 14.9; S, 11.4%.

Compound **116** of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXI] with $SOCl_2$ and 4-aminopyridine gave *N*-(4-pyridyl)-2-chloro-1-methylindole-3-carboxamide [XXII: R_6 = H, R_7 = 4-pyridyl] (61% yield); mp ($CHCl_3$/light petroleum) 220-223°C.

^1H NMR ($(CD_3)_2SO$): δ 10.28 (1H, br, CONH), 8.47 (2H, d, J = 6.1 Hz, H-2',6'), 7.82 (1H, d, J = 7.5 Hz, H-4), 7.72 (2H, d, J = 6.1 Hz, H-3',5'), 7.63 (1H, d, J = 8.0 Hz, H-7), 7.33 (1H, dd, J = 8.0, 7.6 Hz, H-6),

7.25 (1H, dd, J = 7.6, 7.5 Hz, H-5), 3.84 (3H, s, N-CH_3).

^{13}C NMR: δ 162.03 (CONH), 150.16 (d), 145.81 (s), 134.98 (s), 127.50 (s), 124.49 (s), 122.81 (d), 121.54 (d), 119.59 (d), 113.50 (d), 110.47 (d), 107.60 (s), 30.11 (N-CH_3).

Analysis calculated for $C_{15}H_{12}ClN_3O$ requires:
C, 63.1; H, 4.2; N, 14.7%.
Found: C, 62.8; H, 3.9; N, 14.6%.

Reaction of this with MeSLi as above gave 2,2'-dithiobis[*N*-(4-pyridyl)-1-methylindolyl-3-carboxamide] (**116**) [V: R_1 = H, R_2 = CONH-4-pyridyl, R_3 = Me] (53% yield); mp 226-229°C (dec).

^1H NMR ($(CD_3)_2SO$): δ 14.46 (1H, s, CONH), 8.51 (2H, d, J = 7.0 Hz, H-2',6'), 8.13 (2H, d, J = 7.0 Hz, H-3',5'), 8.05 (1H, d, J = 7.9 Hz, H-4), 7.16 (1H, d, J = 8.1 Hz, H-7), 7.00 (2H, m, H-5,6), 3.68 (3H, s, N-CH_3).

^{13}C NMR: δ 165.13 (s), 164.33 (CONH), 153.80 (s), 141.35 (d), 137.26 (s), 128.35 (s), 120.30 (d), 119.97 (d), 118.52 (d), 112.83 (d), 107.66 (d), 104.06 (s), 29.37 (N-CH_3).

Analysis calculated for $C_{30}H_{24}N_6O_2S_2$ requires:
C, 62.8; H, 4.4; N, 14.6; S, 11.2%.
Found: C, 62.4; H, 4.9; N, 14.5; S, 11.4%.

Compound 117 of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXI] with $SOCl_2$ and 3-aminopyridine gave *N*-(3-pyridyl)-2-chloro-1-methylindole-3-carboxamide [XXII: R_7 = H, R_8 = 3-pyridyl] (86% yield); mp (EtOAc/light petroleum) 175-177°C.

^1H NMR ($(CD_3)_2SO$): δ 10.13 (1H, s, CONH), 8.90 (1H, d, J = 2.4 Hz, H-2'), 8.30 (1H, dd, J = 4.7, 1.4 Hz,

H-6'), 8.18 (1H, ddd, J = 4.5, 2.4, 1.4 Hz, H-4'), 7.84 (1H, d, J = 7.9 Hz, H-4), 7.63 (1H, d, J = 8.2 Hz, H-7), 7.40 (1H, dd, J = 4.7, 4.5 Hz, H-5'), 7.32 (1H, dd, J = 8.2, 7.7 Hz, H-6), 7.25 (1H, dd, J = 7.9, 7.7 Hz, H-5), 3.84 (3H, s, N-CH_3).

^{13}C NMR: δ 161.71 (CONH), 144.11 (d), 141.38 (d), 135.85 (s), 134.98 (s), 127.15 (s), 126.62 (d), 124.51 (s), 123.46 (d), 122.74 (d), 121.43 (d), 119.70 (d), 110.43 (d), 107.69 (s), 30.09 (N-CH_3).

Analysis calculated for $C_{15}H_{12}ClN_3O$ requires:
C, 63.1; H, 4.1; N, 14.3; Cl, 13.6%.
Found: C, 63.2; H, 4.2; N, 14.9; Cl, 12.4%.

Treatment of this with MeSLi as above gave 2,2'-dithiobis[*N*-(3-pyridyl) 1-methylindolyl-3-carboxamide] (117) [V: R_1 = H, R_2 = CONH-3-pyridyl, R_3 = Me] (71% yield); mp 257-260°C.

^{1}H NMR ($(CD_3)_2SO$): δ 13.82 (1H, s, CONH), 9.53 (1H, d, J = 1.6 Hz, H-2'), 8.44 (2H, m, H-4',6'), 8.05 (1H, d, J = 8.0 Hz, H-4), 7.91 (1H, dd, J = 4.6, 4.5 Hz, H-5'), 7.14 (1H, d, J = 8.1 Hz, H-7), 6.96 (2H, m, H-5',6'), 3.67 (3H, s, N-CH_3).

^{13}C NMR: δ 164.76 (CONH), 162.70 (s), 140.01 (s), 136.97 (s), 134.17 (d), 132.51 (d), 131.06 (d), 128.44 (s), 127.08 (d), 119.90 (d), 119.45 (d), 118.39 (d), 107.50 (d), 103.89 (s), 29.25. (N-CH_3).

Analysis calculated for $C_{30}H_{24}N_6O_2S_2$ requires:
C, 63.8; H, 4.3; N, 14.9; S, 11.4%.
Found: C, 63.5; H, 4.9; N, 14.8; S, 11.1%.

Compound 118 of Table 1

Treatment of 2-chloro-1-methylindole-3-carboxamide [XXII: R_7 = R_8 = H] (Andreani A, Rambaldi M, J. Het. Chem. 1988;25:1519-1523) with MeSLi as above gave 2,2'-dithiobis[1-methylindolyl-3-carboxamide] (118)

[V: R_1 = H, R_2 = $CONH_2$, R_3 = Me] (71% yield); mp 186-188°C.

^{1}H NMR ($(CD_3)_2SO$): δ 7.99 (1H, d, J = 7.9 Hz, H-4), 7.52 (1H, d, J = 8.3 Hz, H-7), 7.33 (1H, dd, J = 8.3, 7.2 Hz, H-6), 7.25-7.11 (3H, m, H-5 and $CONH_2$), 3.48 (3H, s, N-CH_3).

^{13}C NMR: δ 164.76 ($CONH_2$), 137.56 (s), 129.35 (s), 125.51 (s), 124.37 (d), 121.58 (d), 121.23 (d), 117.77 (s), 110.74 (d), 29.82 (N-CH_3).

Analysis calculated for $C_{20}H_{18}N_4O_2S_2 \cdot 0.5H_2O$ requires:
C, 57.3; H, 4.6; N, 13.4; S, 15.3%.
Found: C, 57.7; H, 4.5; N, 13.5; S, 15.4%.

Compound 119 of Table 1

Treatment of *N,N*-dimethyl 2-chloro-1-methylindole-3-carboxamide [XXII: R_7 = R_8 = Me] (Bergman J, Carlsson R, Sjöberg B, J. Het. Chem. 1977;14:1123-1134) with MeSLi as above gave 2,2'-dithiobis[*N,N*-dimethyl-1-methylindolyl-3-carboxamide] (119) [V: R_1 = H, R_2 = $CONMe_2$, R_3 = Me]. Chromatography on silica gel, eluting with EtOAc, followed by crystallization from EtOAc/light petroleum gave pure material (54% yield); mp 96-102°C.

^{1}H NMR ($CDCl_3$): δ 7.43 (1H, d, J = 8.0 Hz, H-4), 7.31 (2H, m, H-6,7), 7.15 (1H, m, H-5), 3.64 (3H, s, N-CH_3), 2.91, 2.62 (2x3H, 2xbr, $N(CH_3)_2$).

^{13}C NMR: δ 165.89 ($CONMe_2$), 138.06 (s), 128.51 (s), 125.04 (s), 124.47 (d), 121.15 (d), 120.59 (d), 120.19 (s), 110.19 (d), 38.65 ($N(CH_3)_2$), 34.84 ($N(CH_3)_2$), 30.23 (N-CH_3).

Analysis calculated for $C_{24}H_{26}N_4O_2S_2 \cdot 0.5H_2O$ requires:
C, 60.6; H, 5.7; N, 11.7%.
Found: C, 60.3; H, 5.8; N, 11.2%.

Analysis calculated for $C_{24}H_{27}N_4S_2O_2$ requires:
$[M + H]^+$ 467.1575.
Found: $[M + H]^+$ 467.1559 (FAB mass spectrum).

Compound 120 of Table 1

A mixture of 2-chloroindole-3-carboxaldehyde (7.0 g, 36 mmol) was reacted with a slight excess of hydroxylamine hydrochloride and pyridine in refluxing EtOH for 1 hour, to give the crude oxime (Latrell R, Bartmann W, Musif J, Granzer E, German Patent 2,707,268, 31 Aug 1978, Chem. Abstr. 1978;89:179858y). A solution of this in Ac_2O (100 mL) was heated under reflux for 1 hour, cooled, and stirred with water (700 mL). The precipitated solid was collected, washed with water, and crystallized from aqueous MeOH to give 2-chloro-1*H*-indole-3-carbonitrile (3.7 g, 58%); mp 177-180°C.
^1H NMR ($(CD_3)_2SO$): δ 13.23 (1H, s, NH), 7.60 (1H, d, J = 7.5 Hz, ArH), 7.50 (1H, d, J = 7.9 Hz, ArH), 7.34 (1H, t, J = 7.5 H, ArH), 7.29 (1H, t, J = 7.3 Hz, ArH).
^{13}C NMR: δ 134.0, 131.5, 126.2, 114.1 (C), 123.8, 122.3, 117.9, 112.3 (CH), 83.8 (CN).
Analysis calculated for $C_9H_5ClN_2$ requires:
C, 61.2; H, 2.9; N, 15.9%.
Found: C, 61.2; H, 2.7; N, 15.9%.

A solution of the above nitrile (2.5 g, 14 mmol) in Me_2CO was treated with a slight excess of MeI and K_2CO_3 under reflux for 1 hour. Crystallization of the crude product from hexane gave 2-chloro-1-methylindole-3-carbonitrile (1.88 g, 70%); mp 112-114°C.
^1H NMR ($CDCl_3$): δ 7.61-7.55 (1H, m, ArH), 7.34-7.21 (3H, m, ArH), 3.74 (3H, s, CH_3).
^{13}C NMR: δ 135.0, 133.4, 126.0, 114.1 (C), 123.9, 122.7, 118.8, 110.1 (CH), 85.2 (CN).

Analysis calculated for $C_{10}H_7ClN_2$ requires:
C, 63.0; H, 3.7; N, 14.7%.
Found: C, 63.0; H, 3.6; N, 14.7%.

Treatment of this with MeSLi as above gave 2,2'-dithiobis(2-chloro-1-methylindole-3-carbonitrile) (120) [V: R_1 = H, R_2 = CN, R_3 = Me] (53% yield); mp 205-207°C.

^{1}H NMR ($(CD_3)_2SO$): δ 7.69 (1H, d, J = 8.3 Hz, H-4), 7.51 (1H, d, J = 8.0 Hz, H-7), 7.42 (1H, dd, J = 8.0, 7.3 Hz, H-6), 7.28 (1H, dd, J = 8.3, 7.3 Hz, H-5), 3.82 (3H, s, N-CH_3).

Analysis calculated for $C_{20}H_{14}N_4S_2$ requires:
C, 64.2; H, 3.8; N, 15.0; S, 17.1%.
Found: C, 64.2; H, 3.8; N, 15.1; S, 17.7%.

Compound 121 of Table 1

3-Acetyl-2-chloro-1-methylindole was prepared by the reported method (Coppola GM, Hardtmann GE, J. Het. Chem. 1977;14:117-1118). This was reacted with MeSLi as above gave 3-acetyl-1-methyl-2-indolinethione [XV: R_5 = Me] (66% yield); mp 180°C.

^{1}H NMR ($(CD_3)_2SO$): δ 15.60 (1H, br, SH), 7.64 (1H, d, J = 6.5 Hz, H-4), 7.39 (1H, d, J = 7.6 Hz, H-7), 7.32 (1H, dd, J = 7.6, 7.3 Hz, H-6), 7.24 (1H, dd, J = 7.3, 6.5 Hz, H-5), 3.65 (3H, s, N-CH_3), 2.66 (3H, s, $COCH_3$).

^{13}C NMR: δ 178.29 ($COCH_3$), 140.56 (s), 125.21 (d), 124.67 (s), 123.27 (d), 120.60 (d), 111.31 (s), 109.99 (d), 29.31 (N-CH_3), 22.44 ($COCH_3$).

Analysis calculated for $C_9H_5ClN_2$ requires:
C, 61.2; H, 2.9; N, 15.9%.
Found: C, 61.2; H, 2.7; N, 15.9%.

A solution of the above thione (0.10 g, 0.49 mmol) in MeOH/EtOAc (1:9) (10 mL) was stirred vigorously with 30% H_2O_2 (0.20 mL) for 4 hours. The solution was

concentrated to a volume of 0.5 mL, and the orange precipitate was filtered off and washed well with MeOH to give 2,2'-dithiobis(3-acetyl-1-methylindole) (121) [V: R_1 = H, R_2 = COMe, R_3 = Me] (100% yield); mp 178.5-179.5°C.

^{1}H NMR ($(CD_3)_2SO$): δ 8.14 (1H, d, J = 8.1 Hz, H-4), 7.62 (1H, d, J = 8.3 Hz, H-7), 7.39 (1H, dd, J = 8.3, 7.3 Hz, H-6), 7.27 (1H, dd, J = 8.1, 7.3 Hz, H-5), 3.75 (3H, s, N-CH_3), 2.00 (3H, s, $COCH_3$).

^{13}C NMR: δ 192.56 ($COCH_3$), 137.65 (s), 133.73 (s), 125.41 (s), 124.79 (d), 122.73 (d), 121.95 (d), 121.43 (s), 110.92 (d), 30.34 ($COCH_3$), 29.43 (N-CH_3).

Analysis calculated for $C_{22}H_{20}N_2O_2S_2 \cdot 0.5H_2O$ requires:
C, 63.3; H, 5.1; N, 6.7%.
Found: C, 63.7; H, 4.7; N, 6.8%.

Compound 122 of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXI] with $SOCl_2$ and 2-aminopyridine gave *N*-(2'-pyridyl)-2-chloro-1-methylindole-3-carboxamide [XXII: R_6 = H, R_7 = 2-pyridyl] (42% yield); mp (light petroleum) 123°C.

^{1}H NMR ($CDCl_3$): δ 8.85 (1H, s, CONH), 8.41 (1H, d, J = 8.4 Hz, H-4), 8.30 (2H, m), 7.72 (1H, m), 7.28 (3H, m), 7.02 (1H, dd, J = 7.2, 4.9 Hz), 3.74 (3H, s, N-CH_3).

^{13}C NMR: δ 161.58 (CONH), 151.85 (s), 147.92 (d), 138.27 (d), 135.46 (s), 127.22 (s), 125.84 (s), 123.45 (d), 122.48 (d), 121.16 (d), 119.47 (d), 114.25 (d), 109.44 (d), 106.59 (s), 30.21 (N-CH_3).

Analysis calculated for $C_{15}H_{12}ClN_3O$ requires:
C, 63.1; H, 4.2; N, 14.7%.
Found: C, 62.9; H, 4.2; N, 14.4%.

Treatment of this with MeSLi as above gave 2,2'-dithiobis[*N*-(2'-pyridyl)-1-methylindole-3-carboxamide] (**122**) [V: R_1 = H, R_2 = CONH-2-pyridyl, R_3 = Me] (68% yield); mp 270-272°C (dec).

^{1}H NMR ($(CD_3)_2SO$): δ 14.93 (1H, br, CONH), 8.32 (1H, d, J = 6.0 Hz), 8.25 (1H, dd, J = 8.3, 7.7 Hz), 8.02 (1H, dd, J = 7.4, 3.7 Hz), 7.57 (1H, d, J = 8.7 Hz), 7.35 (1H, t, J = 6.6 Hz), 7.21 (1H, dd, J = 5.1, 3.0 Hz), 7.04 (2H, m), 3.69 (3H, s, N-CH_3).

^{13}C NMR: δ 166.48 (s), 165.41 (CONH), 149.16 (s), 145.34 (d), 137.66 (s), 137.49 (s), 127.89 (s), 120.66 (d), 120.44 (d), 118.32 (d), 117.55 (d), 115.32 (d), 107.96 (d), 102.69 (s), 29.40 (N-CH_3).

Analysis calculated for $C_{30}H_{24}N_6O_2S_2 \cdot 0.25H_2O$ requires:
C, 63.3; H, 4.3; N, 14.8; S, 11.3%.
Found: C, 63.2; H, 4.5; N, 14.8; S, 11.7%.

Compound **123** of Table 1

Similar treatment of 1-methyl-2-indolinethione [IV: R_1,R_2 = H, R_3 = CH_3] with the acyl azide derived from 2-furoic acid gave 3-(2-furoyl)-1-methyl-2-indolinethione [IV: R_1 = H, R_2 = CO(2-furyl); R_3 = Me] (85% yield); mp 113.5°C.

^{1}H NMR ($(CD_3)_2SO$): δ 15.90 (1H, br, SH), 8.28 (1H, d, J = 1.6 Hz, H-5'), 7.97 (1H, d, J = 8.0 Hz, H-4), 7.56 (1H, d, J = 3.6 Hz, H-3'), 7.46 (1H, d, J = 8.0 Hz, H-7), 7.37 (1H, dd, J = 8.0, 7.4 Hz, H-6), 7.21 (1H, dd, J = 8.0, 7.4 Hz, H-5), 6.94 (1H, dd, J = 3.6, 1.6 Hz, H-4'), 3.72 (3H, s, N-CH_3).

^{13}C NMR: δ 180.09 (CS), 160.65 (CO), 147.95 (d), 147.27 (s), 140.92 (s), 126.05 (d), 123.26 (s), 123.12 (d), 121.04 (d), 119.19 (d), 113.22 (d), 110.11 (d), 109.64 (s), 29.79 (N-CH_3).

Analysis calculated for $C_{14}H_{11}NO_2S$ requires:
C, 65.3; H, 4.4; N, 5.7; S, 12.7%.
Found: C, 65.4; H, 4,3; N, 5.4; S, 12.5%.

Reaction of the above compound with I_2 as described above gave 2,2'-dithiobis[3-(2-furoyl)-1-methylindole] (**123**) [V: R_1 = H; R_2 = CO(2-furyl); R_3 = Me] (85% yield); mp 175-176.5°C.

^{1}H NMR ($CDCl_3$): δ 7.47 (1H, d, J = 8.1 Hz, H-4), 7.33 (1H, dd, J = 1.6, 0.7 Hz, H-5'), 7.23 (1H, dd, J = 8.1, 7.8 Hz, H-6), 7.21 (1H, d, J = 8.1 Hz, H-7), 7.09 (1H, dd, J = 8.1, 7.9 Hz, H-5), 6.63 (1H, dd, J = 3.6, 0.7 Hz, H-3'), 6.23 (1H, dd, J = 3.6, 1.6 Hz, H-4'), 3.73 (3H, s, NCH_3).

^{13}C NMR: δ 177.09 (CO), 152.55 (s), 145.91 (d), 138.18, 131.32, 125.80 (3xs), 124.72 (d), 123.60 (s), 121.73, 121.12, 119.16, 111.91, 110.06 (5xd), 30.54 (NCH_3).

Analysis calculated for $C_{28}H_{20}N_2O_4S_2 \cdot 0.5H_2O$ requires:
Found: C, 64.4; H, 4.1; N, 5.4; S, 12.3%.
C, 64.7; H, 4.1; N, 5.6; S, 12.4%.

Compound 124 of Table 1

Similar treatment of 1-methyl-2-indolinethione [IV: R_1,R_2 = H, R_3 = CH_3] with the isocyanate derived from thiophene-2-carboxylic acid gave 2,2'-dithiobis[*N*-(2-thienyl)-1-methylindole-3-carboxamide] (**124**) [V: R_1 = H, R_2 = CONHfuryl, R_3 = Me] (21% yield; mp 183°C (dec).

^{1}H NMR ($(CD_3)_2SO$): δ 11.26 (1H, s, CONH), 7.93 (1H, d, *J* = 8.0 Hz, H-4), 7.62 (1H, d, *J* = 8.3 Hz, H-7), 7.34 (1H, dd, *J* = 8.3, 7.4 Hz, H-6), 7.24 (1H, dd, *J* = 8.0, 7.4 Hz, H-5), 7.05 (1H, dd, *J* = 5.3, 3.6 Hz, H-4'), 6.94 (1H, d, *J* = 5.3 Hz, H-5'), 6.41 (1H, d, *J* = 3.6 Hz, H-3'), 3.95 (3H, s, NCH_3).

^{13}C NMR: δ 160.10 (CONH), 139.86 (s), 137.81 (s), 136.86 (s), 125.19 (s), 123.96 (d), 123.69 (d), 121.28 (d), 120.54 (d), 116.85 (d), 114.73 (s), 111.20 (d), 110.77 (d), 30.54 (N-CH_3).

Analysis calculated for $C_{28}H_{22}N_4O_2S_4 \cdot H_2O$ requires:
C, 57.6; H, 4.0; N, 9.6%.
Found: C, 57.6; H, 4.1; N, 10.0%.

EXAMPLE J

Preparation of Compound 125 of Table 1 by the Method Outlined in Scheme 9

Reaction of 3-chlorocarbonyl-1-(phenylsulfonyl)-indole [XXIII] (Ketcha DM, Gribble GW, J. Org. Chem. 1985;50:5451-5457) with an excess of benzylamine in CH_2Cl_2) (method of Ketcha and Gribble) gave *N*-benzyl-1-(phenylsulfonyl)indole-3-carboxamide [XXIV: R_8 = CH_2Ph]; mp (MeOH) 188-189°C.

^1H NMR ($CDCl_3$): δ 8.05 (1H, s, H-2), 8.03-7.86 (4H, m, ArH), 7.56-7.26 (10H, m, ArH), 6.43 (1H, m, NH), 4.64 (2H, d, *J* = 5.7 Hz, CH_2).

Analysis calculated for $C_{22}H_{18}N_2O_3S$ requires:
C, 67.7; H, 4.5; N, 7.2; S, 8.2%.
Found: C, 67.4; H, 4.8; N, 7.1; S, 8.2%.

A solution of the above *N*-benzyl-1-(phenylsulfonyl)indole-3-carboxamide [XXIV: R_8 = CH_2Ph] (4.2 g, 11 mmol) in dry THF (200 mL) was treated at -78°C with a solution of 2.5 M n-BuLi in hexanes (9.1 mL, 23 mmol), and the stirred mixture was allowed to warm to -20°C for 15 minutes, before being recooled to -78°C, when it was treated with methyldisulfide (2.5 mL, 28 mmol). The mixture was allowed to warm to 20°C, then quenched with water (25 mL). Volatiles were removed under reduced pressure, and the residue was extracted with EtOAc. Workup of the organic layer gave

a crude product. This was dissolved in MeOH (300 mL), mixed with a solution of K_2CO_3 (6.9 g, 50 mmol) in water (125 mL), and heated under gentle reflux under N_2 for 2 hours to ensure complete hydrolysis of the phenylsulfonyl group (J. Org. Chem. 1985;50:5451-5457). MeOH was removed under reduced pressure, and the residue was diluted with water and extracted with CH_2Cl_2. Chromatography of the resulting oil on Al_2O_3 (eluting with CH_2Cl_2) gave *N*-benzyl-2-(methylthio)-indole-3-carboxamide [XXV: $R_8 = CH_2Ph$] (2.8 g, 88% yield) as an oil.

^1H NMR ($CDCl_3$): δ 10.65 (1H, s, H-1), 8.29 (d, J = 5.1 Hz, H-4), 7.87 (1H, t, J = 5.6 Hz, CONH), 7.34-7.08 (8H, m, ArH), 4.73 (2H, d, J = 5.6 Hz, CH_2), 2.33 (3H, s, SMe).

^{13}C NMR ($CDCl_3$): δ 165.6 (C=O), 138.5, 136.4, 133.1 and 110.8 (C), 128.5, 127.2, 127.1, 122.9, 121.4, 126.8 and 111.2 (CH), 43.2 (CH_2), 18.5 (CH_3).

HREIMS calculated for $C_{17}H_{16}N_2OS$:
296.0983.
Found: 296.0985.

A solution of the above *N*-benzyl-2-(methylthio)-indole-3-carboxamide [XXV: R = CH_2Ph] (0.85 g, 2.87 mmol) in DMA (5 mL) was added under N_2 to a stirred suspension of MeSLi (0.93 g, 17.2 mmol) in DMA (10 mL). After warming at 80°C for 6 hours, the mixture was acidified with 3N HCl, extracted with CH_2Cl_2, and worked up. Traces of DMA were removed under high vacuum, and the residue was dissolved in MeOH (15 mL) and treated dropwise with H_2O_2 (0.5 mL of 30% solution). After chilling at -30°C overnight, the precipitate was filtered off to give 2,2'-dithiobis[*N*-benzylindolyl-3-carboxamide] (125) [V: $R_1 = R_3 = H$, $R_2 = CONHCH_2Ph$], (74%); mp 203-205°C.

^{1}H NMR ($(CD_3)_2SO$): δ 12.97 (1H, s, NH), 8.48 (1H, t, J = 5.7 Hz, CONHCH$_2$), 7.86 (1H, d, J = 8.2 Hz, H-4), 7.40 (2H, d, J = 8.3 Hz, H-2',6'), 7.34 (3H, dd, J = 8.3, 8.2 Hz, H-7,3',5'), 7.25 (1H, t, J = 8.2 Hz, H-4'), 7.20-7.10 (2H, m, H-5,6), 4.56 (2H, d, J = 5.7 Hz, CONHCH$_2$).

^{13}C NMR: δ 164.71 (CONH), 139.77 (s), 136.69 (s), 135.30 (s), 128.16 (d), 127.15 (d), 126.56 (d), 124.44 (s), 122.63 (d), 120.78 (d), 119.25 (d), 111.60 (d), 110.54 (s), 42.62 (CONHCH$_2$).

Analysis calculated for $C_{32}N_{26}N_4O_2S_2$ requires:
C, 68.3; H, 4.7; N, 10.0; S, 11.4%.
Found: C, 68.0; H, 4.8; N, 9.9; S, 11.2%.

Compound 126 of Table 1

Reaction of 3-chlorocarbonyl-1-(phenylsulfonyl)-indole [XXIII] with an excess of aniline as above gave *N*-phenyl-1-(phenylsulfonyl)indole-3-carboxamide [XXIV: R_8 = Ph]; mp (MeOH) 220-222.5°C.

^{1}H NMR: δ ($CDCl_3$) 8.18 (1H, s, H-2), 8.12 (1H, d, J = 7.8 Hz, H-4), 7.99 (1H, d, J = 8.3 Hz, H-7), 7.91 (2H, d, J = 7.9 Hz, ArH), 7.90 (1H, m, NH), 7.65 (2H, d, J = 8.4 Hz, ArH), 7. 57 (1H, t, J = 7.8 Hz, ArH), 7.45 (2H, t, J = 7.8 Hz, ArH), 7.41-7.33 (4H, m, ArH), 7.15 (1H, t, J = 7.4 Hz, H-5).

Analysis calculated for $C_{21}H_{18}N_2O_3S$ requires:
C, 67.0; H, 4.3; N, 7.4; S, 8.5%.
Found: C, 66.9; H, 4.4; N, 7.3; S, 8.5%.

Treatment of this with n-BuLi/methyldisulfide as above gave 2-(methylthio)-*N*-phenylindole-3-carboxamide [XXV: R_8 = Ph] (81%) as an oil.

^{1}H NMR ($CDCl_3$): δ 10.19 (1H, s, H-1), 9.59 (1H, s, CONH), 8.47 (1H, d, J = 6.8 Hz, H-4), 7.80 (2H, d,

J = 8.5 Hz, ArH), 7.43-7.35 (3H, m, ArH), 7.28-7.16 (3H, m, ArH), 2.51 (3H, s, SCH_3).

^{13}C NMR ($CDCl_3$): δ 163.5 (CO), 138.2, 136.1, 132.5, 127.3, 111.2 (CH), 19.1 (CH_3).

HREIMS calculated for $C_{16}H_{14}N_2OS$:
282.0827
Found: 282.0827.

Treatment of this with MeSLi as above gave 2,2'-dithiobis[*N*-phenylindolyl-3-carboxamide] (126) [V: R_1 = R_3 = H, R_2 = CONHPh], (67%); mp 220-223°C.

^1H NMR ($(CD_3)_2SO$): δ 12.73 (1H, s, NH), 9.88 (1H, s, CONH), 7.81 (1H, d, J = 7.9 Hz, H-4), 7.69 (2H, d, J = 8.4 Hz, H-2',6'), 7.46 (1H, d, J = 7.7 Hz, H-7), 7.34 (2H, dd, J = 8.4, 8.3 Hz, H-3',5'), 7.24 (1H, dd, J = 7.7, 7.7 Hz, H-6), 7.17 (1H, dd, J = 7.9, 7.7 Hz, H-5), 7.10 (1H, dd, J = 8.3 Hz, H-4').

^{13}C NMR: δ 163.27 (CONH), 138.89 (s), 136.73 (s), 133.94 (s), 128.53 (d), 125.12 (s), 123.49 (d), 123.17 (d), 120.99 (d), 120.32 (d), 119.97 (d), 112.89 (s), 111.67 (d).

Analysis calculated for $C_{30}H_{22}N_4O_2S_2$ requires:
C, 67.4; H, 4.2; N, 10.5; S, 12.0%.
Found: C, 67.1; H, 4.3; N, 10.6; S, 12.0%.

Compound **127** of Table 1

Reaction of 3-chlorocarbonyl-1-(phenylsulfonyl)-indole [XXIII] with an excess of methylamine as above gave *N*-methyl-1-(phenylsulfonyl)indole-3-carboxamide [XXIV: R_8 = Me]; mp (MeOH) 192.5-195°C.

^1H NMR ($CDCl_3$): δ 8.06 (1H, s, H-2), 8.03-7.84 (4H, m, ArH) 7.53-7.26 (5H, m, ArH), 6.37 (1H, m, NH), 2.99 (d, J = 4.9 Hz, CH_3).

Analysis calculated for $C_{16}H_{14}N_2O_3S$ requires:
C, 61.1, H, 4.5; N, 8.9; S, 10.2%.
Found: C, 61.1; H, 4.7; N, 8.9; S, 10.0%.

Treatment of this with n-BuLi/methyldisulfide as above gave *N*-methyl-2-(methylthio)indole-3-carboxamide [XXV: R_8 = Me] (95%); mp (hexane-CH_2Cl_2) 138.5-139.5°C.

^{1}H NMR ($CDCl_3$): δ 10.31 (1H, s, H-1), 8.35-8.26 (1H, m, H-4), 7.44 (1H, t, J = 4.8 Hz, NH), 7.38-7.30 (1H, m, ArH), 7.19-7.11 (2H, m, ArH), 3.06 (3H, d, J = 4.8 Hz, CH_3), 2.49 (3H, s, SCH_3).

^{13}C NMR ($CDCl_3$): δ 166.4 (CO), 136.4, 132.4, 127.4 and 111.7 (C), 123.1, 121.5, 121.2, 111.1 (CH), 26.3 and 18.9 (CH_3).

Analysis calculated for $C_{11}H_{12}N_2OS$ requires:
C, 60.0; H, 5.5; N, 12.7; S, 14.6%.
Found: C, 59.8; H, 5.7; N, 12.7; S, 14.5%.

Treatment of this with MeSLi as above gave 2,2'-dithiobis[*N*-methylindolinyl-3-carboxamide] (127) [V: R_1 = R_3 = H, R_2 = CONHMe], (57% yield); mp 232-236°C (dec).

^{1}H NMR ($(CD_3)_2SO$): δ 12.94 (1H, s, NH), 7.85 (1H, br, CONH), 7.81 (1H, d, J = 8.0 Hz, H-4), 7.46 (1H, d, J = 8.0 Hz, H-7), 7.20 (1H, dd, J = 8.0, 7.7 Hz, H-6), 7.14 (1H, dd, J = 8.0, 7.7 Hz, H-5), 2.88 (3H, d, J = 4.5 Hz, $CONHCH_3$).

^{13}H NMR: δ 165.20 (CONH), 136.70 (s), 134.76 (s), 124.47 (s), 122.61 (d), 120.71 (d), 119.55 (d), 111.55 (d), 111.02 (s), 26.22 ($CONHCH_3$).

Analysis calculated for $C_{20}H_{18}N_4O_2S_2$ requires:
C, 58.5; H, 4.4; N, 13.7; S, 15.6%.
Found: C, 58.4; H, 4.7; N, 13.6; S, 15.4%.

Compound 128 of Table 1

A solution of 2-(methylthio)-*N*-phenyl-1*H*-indole-3-carboxamide [XXV: R_8 = H] (1.8 g, 6.4 mmol) in EtOH (400 mL) was treated with 3-(dimethylamino)propyl chloride hydrochloride (10.0 g, 64 mmol) and K_2CO_3 (13 g, 96 mmol) and heated under reflux for 3 hours. A further 10 equivalents of the reagents were then added, and the mixture was heated under reflux for a further 48 hours. EtOH was removed under reduced pressure, and the residue was diluted with water to give crude product. This was chromatographed on alumina, eluting with CH_2Cl_2 containing 0.2% MeOH, to give 1-[3-(dimethylamino)propyl]-2-(methylthio)-*N*-phenyl-1*H*-indole-3-carboxamide [XXVI: R_8 = H, R_9 = $(CH_2)_3NMe_2$] (0.49 g, 21%) as an oil.

^{1}H NMR ($CDCl_3$): δ 9.93 (1H, s, NH), 8.54 (1H, d, J = 7.8 Hz, H-4), 7.74 (2H, d, J = 8.6 Hz, H-2',6'), 7.42-7.24 (5H, m, ArH), 7.11 (1H, t, J = 7.4 Hz, ArH), 4.46 (2H, t, J = 7.4 Hz, 1-CH_2), 2.47 (3H, s, SCH_3), 2.37 (2H, t, J = 6.9 Hz, CH_2N), 2.27 (6H, s, $N(CH_3)_2$), 1.97 (2H, dxt, J = 7.4, 6.9 Hz, $CH_2CH_2CH_2$).

^{13}C NMR: δ 162.6 (CO), 138.8, 136.7, 131.4, 127.5, 114.1 (C), 129.0, 124.1, 123.7, 122.8, 122.1, 119.8, 110.0 (CH), 56.5, 42.0, 28.3 (CH_2), 45.3 ($N(CH_3)_2$), 21.1 (SCH_3).

Analysis calculated for $C_{21}H_{25}N_3O_8$ requires:

$[M + H^+]$ = 368.1797.

HRFABMS Found: $[M + H^+]$ = 368.1812.

This was treated with MeSLi at 80°C for 8 hours as above. Water was added, the mixture was washed with CH_2Cl_2, and the aqueous portion was carefully neutralized with 3N HCl and extracted with CH_2Cl_2. This extract was worked up to give an oil which was dissolved in MeOH and treated dropwise at room

temperature with a saturated solution of I_2 in CH_2Cl_2 until no starting material was evident on TLC analysis. The reaction mixture was absorbed directly onto silica and chromatographed. MeOH/EtOAc (1:9) eluted foreruns, while MeOH/EtOAc (1:9) containing a trace of concentrated NH_4OH gave 2,2'-dithiobis[1-{3-(dimethylamino)}propyl)-*N*-phenyl-1*H*-indole-3-carboxamide] (**128**) [V: R_1 = H, R_2 = CONHPh, R_3 = $(CH_2)_3NMe_2$] (10% yield) as a yellow foam.

^1H NMR (CD_3OD): δ 8.19 (1H, d, J = 7.3 Hz, H-4), 7.64 (1H, d, J = 7.5 Hz, H-7), 7.30-7.20 (3H, m, ArH), 7.10-6.95 (4H, m, ArH), 4.41 (2H, t, J = 6.2 Hz, CH_2N), 2.74 (2H, t, J = 6.7 Hz, CH_2NMe_2), 2.64 (6H, s, $N(CH_3)_2$), 2.09 (2H, m, $CH_2CH_2CH_2$).

Analysis calculated for $C_{40}H_{45}N_6O_2S_2$ requires:
$[M + H^+]$ = 705.3045.
HRFABMS found: $[M + H^+]$ = 705.3035.

EXAMPLE K

<u>Preparation of Compound 129 of Table 1 by the Method Outlined in Scheme 10</u>

To a stirred 25°C solution of 41 mL (558 mmol) of DMF and 75 mL of dichloromethane was added dropwise a solution of 133.5 g (465 mmol) of $POBr_3$ in 100 mL of dichloromethane at such a rate to maintain a gentle reflux via the exothermic reaction (ca. 1 hour). The resulting thick tan suspension was stirred vigorously for 10 minutes, then treated dropwise over 20 minutes with a solution of 27.38 g (186 mmol) of 1-methyl-2-indolinone [VII: R_1 = H, R_3 = CH_3) in 55 mL of dichloromethane. The mixture was heated at reflux for 3.5 hours, cooled to 25°C, and the supernatant was decanted and concentrated to a thick reddish brown oil. This was combined with the solids above and treated

very cautiously with portionwise addition of ca. 20 g of ice, then with 112 g of 50% (w/w) aqueous NaOH, all the while keeping the temperature between 30-40°C (pH = 3). An additional 20 g of 50% NaOH, then 100 mL of ice water were added, and the precipitate was collected by filtration. The solids were washed well with water, then dried over P_2O_5 to leave 42.6 g of crude bromoaldehyde; mp 92-97°C. The solids were dissolved in ca. 65 mL of dichloromethane and the solution filtered over 165 g of flash silica gel placed in a 600 mL sintered glass funnel. The frit was washed with dichloromethane until all the product had eluted. The combined product fractions were concentrated to leave 34.66 g (78%) of nearly pure 2-bromo-1-methylindole-3-carboxaldehyde [XXVI: R_1 = H, R_3 = CH_3, X = Br]; mp 110-112° which was used directly in the next reaction.

To a vigorously stirred solution of 2.38 g (10 mmol) of 2-bromo-1-methylindole-3-carboxaldehyde [XXVI: R_1 = H, R_3 = CH_3, X = Br], 10 mL of 2-methyl-2-butene, and 40 mL of p-dioxane at 25°C was added dropwise over ca. 15 minutes a solution of 5 g (55 mmol) of sodium chlorite and 5 g (36 mmol) of $NaH_2PO_4 \cdot H_2O$ in 25 mL of water. The solution was maintained at 25°C. After 3.5 hours, the mixture was treated with an additional 2.5 g each of the chlorite and phosphate. After a total reaction time of 24 hours, the mixture was extracted 3 times with dichloromethane, then the aqueous phase was acidified to pH 2 with aqueous HCl, and extracted once more. The combined organic extracts were washed with water, dried, and evaporated to leave a solid residue that was boiled in 2-propanol. After cooling, the solids were collected by filtration, washed with a little

2-propanol, and dried to leave 2.21 g (87%) of 2-bromo-1-methylindole-3-carboxylic acid [XXVII: R_1 = H, R_3 = CH_3, X = Br] as a beige solid; mp ca. 198°C (dec), in 2 crops.

A suspension of 2.54 g (10 mmol) of 2-bromo-1-methylindole-3-carboxylic acid [XXVII: R_1 = H, R_3 = CH_3, X = Br], 2.54 g (10 mmol) of bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 2.78 mL (20 mmol) of triethylamine, and 25 mL of 1,2-dichloroothane was heated at reflux for 1.5 hours. The mixture was cooled and poured into 150 mL 5% aqueous sodium bicarbonate solution and stirred for 30 minutes. The mixture was extracted with dichloromethane (3 times), the combined organic phase washed with water, brine, dried ($MgSO_4$), and concentrated to leave a red oil. The oil was triturated in ethyl acetate:hexanes and the solids were collected by filtration to give 0.95 g of a side product; mp 227-228°C (dec). The filtrate was concentrated to a viscous oil that was dissolved into chloroform and adsorbed into 9 g of flash SiO_2. This was introduced onto a column containing flash SiO_2 and the column was eluted with hexanes:ethyl acetate (95:5). Product fractions were pooled, concentrated, and triturated from isooctane to give 1.96 g (63%) of 2-bromo-1-methylindole-3-carboxylic acid, t-butyl ester [XXVIII: R_1 = H, R_2 = COO-t-butyl, R_3 = CH_3] as a white solid; mp 87-88°C.

Analysis calculated for $C_{14}H_{16}BrNO_2$ requires:
C, 54.21; H, 5.20; N, 4.52; Br, 25.76%.
Found: C, 54.28; H, 5.20; N, 4.49, Br, 25.83%.

An ice-cold suspension of 119 mg (1.5 mmol) of elemental selenium in 2 mL of THF under N_2 was treated dropwise with 1.1 mL of methyl lithium:lithium bromide

complex (1.5 M in ether). The flask was opened to the air and with a brisk stream of N_2, the resultant white suspension was warmed to ca. 85°C to distill off the ether and most of the THF. The residual semi-solid was cooled in an ice bath and diluted with 1.5 mL of DMA followed by 155 mg (0.5 mmol) of 2-bromo-1-methylindole-3-carboxylic acid, t-butyl ester. The resultant solution was stirred at room temperature for 24 hours, cooled to 0°C, then treated with 2 mL of dilute acetic acid. The mixture was diluted with water and extracted with chloroform (3 x 10 mL). The combined extracts were washed with water (4 times), dried (Na_2SO_4), and concentrated to leave a golden solid. The solid was suspended in 2.3 mL of 2:1 v/v HOAc:H_2O and the suspension was treated with 154 mg of $NaBO3 \cdot 4H_2O$, then stirred at 25°C for 30 minutes. The solids were collected by filtration, washed with water, and dried to leave 119 mg (77 %) of 2,2'-diselenobis [1-methyl-1H-indole-3-carboxylic acid, t-butyl ester] (129) [XXIX: R_1 = H, R_2 = COO-t-butyl, R_3 = CH_3]; mp 187-189°C.

^{1}H NMR ($CDCl_3$): ∂ 8.13 (1H, dd, J = 0.7, 7.9 Hz, H-4), 7.31-7.19 (3H, m, ArH), 3.63 (3H, s, NCH_3), 1.44 (9H, s, $C(CH_3)_3$).

Analysis calculated for $C_{28}H_{32}N_2O_4Se_2 \cdot 0.2H_2O$ requires:
C, 54.06; H, 5.25; N, 4.50%.
Found: C, 54.40; H, 5.48; N, 4.11%.

Compound 130 of Table 1

To an ice-cold solution of 4 mL of trifluoroacetic acid under nitrogen was added 420 mg (0.68 mmol) of 2,2'-diselenobis[1-methyl-1H-indole-3-carboxylic acid, t-butyl ester] (101) [XXIX: R_1 = H, R_2 = COO-t-butyl, R_3 = CH_3]. The suspension was maintained at 0°C for

3 hours, then poured into ice water. The solids were collected by filtration, washed well with water, and dried to leave 361 mg of product; mp 165°C (dec). The solids were suspended into 80 mL 10% aqueous NH_4OH and the insolubles were removed by filtration. The filtrate was adjusted to pH 3 with 6N aqueous HCl, and the precipitated solids were collected by filtration, washed with water, and dried to leave 268 mg (78%) of 2,2'-diselenobis[1-methyl-1H-indole-3-carboxylic acid] (130) [XXIX: R_1 = H, R_2 = COOH, R_3 = CH_3]; mp 174°C (dec) as an orange solid.

^{1}H NMR ($(CD_3)_2SO$): ∂ 12.35 (1H, s, $CO_2\underline{H}$), 8.04 (1H, d, J = 7.9 Hz, H-4), 7.56 (1H, d, J = 8.4 Hz, H-7), 7.31-7.20 (2H, m, ArH), 3.63 (3H, s, NCH_3).

Analysis calculated for $C_{20}H_{16}N_2O_4Se_2 \cdot 0.1H_2O$ requires:
C, 47.28; H, 3.21; N, 5.51%.
Found: C, 47.20; H, 3.20; N, 5.12%.

Compound 131 of Table 1

A 25°C suspension of 2.79 g (11 mmol) of 2-bromo-1-methylindole-3-carboxylic acid [XXVII: R_1 = H, R_3 = CH_3, X = Br] in 13 mL of 1,2-dichloroethane was treated dropwise with 2.41 mL (33 mmol) of thionyl chloride. The mixture was heated at 75°C for 2 hours. The solution was concentrated to a solid which was co-evaporated once with dichloromethane. The solid was ice-cooled and treated rapidly with 26 mL of 40% aqueous methylamine. The bath was removed and the suspension was stirred at 25°C for 2 hours. The solids were collected by filtration, washed well with water, and dried at 200 mm/70°C/12 hours over P_2O_5 to leave 2.2 g (75%) of product; mp 154-157°C.

Recrystallization from MeOH provided 1.91 g of pure 2-bromo-1-methylindole-3-N-methylcarboxamide

[XXX: R_1 = H, R_3 = CH_3, R_7 = H, R_8 = CH_3] as a beige solid; mp 159-160°C in three crops.

An ice-cold solution of lithium methyl selenide in 2 mL of DMA, made up as previously described from 237 mg (3 mmol) of elemental Se and 2.2 mL of methyllithium (1.5 M in ether) in 3 mL of THF, was treated with 267 mg (1.0 mmol) of 2-bromo-1-methylindole-3-N-methylcarboxamide [XXX: R_1 = H, R_3 = CH_3, R_7 = H, R_8 = CH_3]. The resultant solution was stirred at room temperature for 3.5 hours, cooled to 0°C, then treated with 5% aqueous HCl. The mixture was extracted with dichloromethane (2 x 10 mL), the combined extracts washed with water (2 times), then concentrated in vacuo to leave an oil that was dissolved in methanol. The solution was ice-cooled and treated with 113 μL of 30% aqueous H_2O_2. After stirring for 10 minutes, the resultant suspension was filtered, and the solids were washed with 2-propanol and dried to leave 183 mg (67%) of 2,2'-diselenobis [N,1-dimethyl-1H-indole-3-carboxamide] (131) [XXIX: R_1 = H, R_2 = $CONHCH_3$, R_3 = CH_3] as a yellow solid; mp 225-230°C (dec).

^{1}H NMR ($CDCl_3$ + $(CD_3)_2SO$): ∂ 7.97 (1H, d, J = 7.9 Hz, H-4), 7.39-7.18 (3H, m, ArH), 6.84 (1H, s, $NHCH_3$), 3.85 (3H, s, indole NCH_3), 2.12 (3H, d, J = 4.5 Hz, $NHCH_3$).

Analysis calculated for $C_{22}H_{22}N_4O_2Se_2 \cdot 0.9H_2O$ requires:
C, 48.17; H, 4.37; N, 10.21%.
Found: C, 48.20; H, 4.22; N, 10.28%.

Compound 132 of Table 1

Similar reaction of 2-chloro-1-methylindole-3-carboxylic acid [XXVII: R_1 = H, R_3 = CH_3, X = Cl] with $SOCl_2$ as described in Example I and reaction of this with 3 equivalents of N,N-diethylethylenediamine

in dichloromethane at 0°C followed by workup gave 2-chloro-1-methylindole-3-N-(2-(diethylamino)ethyl)-carboxamide [XXX: R_1 = H, R_6 = H, R_7 = $(CH_2)_2NEt_2$, X = Cl] as a soft solid in 68% yield, used without further purification.

Treatment of this with lithium methyl selenide as described above gave 2,2'-diselenobis[N-[2-(diethylamino)ethyl]-1-methyl-1H-indole-3-carboxamide] (132) [XXIX: R_1 = H, R_2 = $CONH(CH_2)_2NEt_2$, R_3 = CH_3] (68% yield); mp 128-130°C. Reaction of the free base with excess hydrogen chloride in 2-propanol followed by concentration to an oil and crystallization at 25°C gave the compound as a dihydrochloride salt (18% yield); mp 160-164°C.

^{1}H NMR ($(CD_3)_2SO$): ∂ 10.13 (1H, s, $^+N\underline{H}(CH_2CH_3)_2$), 8.14-8.11 (1H, m, CON<u>H</u>), 7.89 (1H, d, J = 8.2 Hz, H-4), 7.57 (1H, d, J = 8.4 Hz, H-7), 7.34-7.17 (2H, m, ArH), 3.63 (3H, s, $NC\underline{H}_3$), 3.17-3.14 (2H, m, $CONHC\underline{H}_2$), 3.06-3.00 (4H, m, $N(C\underline{H}_2CH_3)_2$), 2.86 (2H, t, J = 6.5 Hz, $CONHCH_2C\underline{H}_2$), 1.16 (6H, t, J = 7.2 Hz, $N(CH_2C\underline{H}_3)_2$).

Analysis calculated for $C_{32}H_{44}N_6O_2Se_2 \cdot 2.0HCl \cdot 1.7H_2O$ requires:

C, 47.67; H, 6.18; N, 10.42; Cl^-, 8.79%.

Found: C, 47.71; H, 6.12; N, 10.35; Cl^-, 8.97%.

<u>Compound 133 of Table 1</u>

A mechanically stirred suspension of 15 g (83.5 mmol) of 2-chloroindole-3-carboxaldehyde [XXVI: R_1 = R_3 = H, X = Cl] (Schule, et al., <u>Arch. Pharm.</u> [Weinheim] 1972;305:523-533), 84 mL of 2-methyl-2-butene, and 200 mL of p-dioxane in an ice bath was treated with a solution of 40 g each of sodium chlorite and sodium dihydrogen phosphate monohydrate in 200 mL of water. The biphasic mixture was then stirred

vigorously at 25°C for 3.5 hours. An additional 16 g each of solid sodium chlorite and sodium dihydrogen phosphate monohydrate was added and the mixture was stirred for another 3.5 hours. The mixture was diluted with 350 mL of ethyl acetate and 200 mL of water. The layers were separated and the aqueous phase was extracted with 300 mL of ethyl acetate. The combined organic extracts were extracted with cold 2% aqueous NaOH (3 x 200 mL). The basic extracts were combined and acidified to pH 4 with 6N aqueous HCl. The precipitated solids were collected by filtration, washed well with water, and air dried overnight. The solids were dissolved in 150 mL of hot acetone and the solution was treated with 65 mL of hexane. After storage at 3°C for 20 hours, the solids were collected by filtration, washed with cold acetone, and dried to leave 7.71 g of pure 2-chloroindole-3-carboxylic acid [XXVII: R_1 = R_3 = H, X = Cl] as an off-white solid; mp 181.5°C (dec). Further processing of the filtrate as above afforded 2.41 g of a second crop; mp 179.5°C (dec). Total yield 10.12 g (62%).

The acid chloride of 2-chloroindole-3-carboxylic acid [XXVII: R_1 = R_3 = H, X = Cl] was made via $SOCl_2$ as described above. Reaction of this with a saturated solution of anhydrous methylamine in THF at 0°C gave 2-chloroindole-3-N-methylcarboxamide [XXX: R_1 = R_3 = H, R_6 = H, R_7 = CH_3, X = Cl]; mp 234-236°C, in 51% yield.

Reaction of this with lithium methyl selenide as described above gave 2,2'-diselenobis[N-methyl-1H-indole-3-carboxamide] (133) [XXIX: R_1 = R_3 = H, R_3 = $CONHCH_3$] (20% yield), mp 272-275°C (decomp). ^1H NMR ($(CD_3)_2SO$): ∂ 12.36 (1H, s, indole NH), 7.83 (1H, d, J = 7.7 Hz, H-4), 7.79 (1H, d, J = 4.1, $N\underline{H}CH_3$),

7.48 (1H, d, J = 7.7 Hz, H-7), 7.16-7.07 (2H, m, ArH), 2.90 (3H, d, J = 4.1 Hz, $NHC\underline{H}_3$).

Analysis calculated for $C_{20}H_{18}N_4O_2Se_2 \cdot 0.9H_2O$ requires:
C, 46.15; H, 3.83; N, 10.76%.
Found: C, 46.08; H, 3.44; N, 10.45%.

Compound 134 of Table 1

The acid chloride of 2-chloroindole-3-carboxylic acid [XXVII: $R_1 = R_3 = H$, X = Cl] was made via $SOCl_2$ as described above. Reaction of this with 3 equivalents of N,N-diethylethylenediamine in ether as described above followed by workup gave 2-chloroindole-3-N-(2-(diethylamino)ethyl)carboxamide [XXX: $R_1 = R_3 = R_6 = H$, $R_7 = (CH_2)NEt_2$, X = Cl]; mp 99-108°C in 38% yield.

^1H NMR ($CDCl_3$): ∂ 11.50 (1H, s, indole NH), 8.19 (1H, d, J = 6.5 Hz, H-4), 7.33 (1H, d, J = 8.4 Hz, H-7), 7.21-7.15 (3H, m, ArH and $CONH$), 3.54 (2H, q, J = 5.3 Hz, $CONHCH_2$), 2.69 (2H, t, J = 6.0 Hz, $CONHCH_2CH_2$), 2.59 (4H, q, J = 7.2 Hz, $N(CH_2CH_3)_2$), 1.05 (6H, t, J = 7.2 Hz, $N(CH_2CH_3)_2$).

Reaction of this with lithium methyl selenide as described above gave 2,2'-diselenobis[N-[2-(diethylamino)ethyl]-1H-indole-3-carboxamide] (134) [XXIX: $R_1 = R_3 = H$, $R_2 = CONH(CH_2)_2NEt_2$] (44% yield); mp 225-226°C (dec). Salt formation as above gave the compound as the dihydrochloride salt (85% yield); mp 257-259°C (dec).

^1H NMR ($(CD_3)_2SO$): ∂ 12.75 (1H, s, indole NH), 10.08 (1H, s, $^+NH(CH_2CH_3)_2$), 8.09 (1H, t, J = 5.7 Hz, $CONH$), 7.93 (1H, d, J = 8.9 Hz, H-4), 7.51 (1H, d, J = 6.8 Hz, H-7), 7.19-7.12 (2H, m, ArH), 3.78-3.73 (2H, m, $CONHCH_2$), 3.32 (2H, t, J = 6.5 Hz, $CONHCH_2CH_2$),

3.29-3.20 (4H, m, $N(C\underline{H}_2CH_3)_2$), 1.26 (6H, t, J = 7.2 Hz, $N(CH_2C\underline{H}_3)_2$).

Analysis calculated for $C_{30}H_{40}N_6O_2Se_2 \cdot 2.0HCl \cdot 1.0H_2O$ requires:

C, 47.07; H, 5.79; N, 10.98; Cl^-, 9.26%.

Found: C, 47.01; H, 5.70; N, 10.56; Cl^-, 8.87%.

Compound 135 of Table 1

A mixture of 2.09 g (10 mmol) of 2-chloroindole-3-N-methylcarboxamide [XXX: $R_1 = R_3 = R_6 = H$, $R_7 = CH_3$, X = Cl], 1.72 g (10 mmol) of 2-diethylaminoethyl chloride hydrochloride (n = 2, Q = Cl, $R_8 = R_9 = Et$), 7.5 g (23 mmol) of anhydrous cesium carbonate, 3 g of activated 3A molecular sieves, and 20 mL of acetone was stirred under nitrogen at 25°C for 16 hours. The mixture was filtered over celite and the filtrate was concentrated to a solid that was partitioned between chloroform and water. The organic phase was dried (Na_2SO_4) and concentrated to a residue that was crystallized from ethyl acetate:hexanes (5:8). The solids were collected and dried to leave 1.43 g of 2-chloro-1-[2-(diethylamino)ethyl]-N-methyl-1H-indole-3-carboxamide [XXX: $R_1 = R_6 = H$, $R_3 = (CH_2)_2NEt_2$, $R_7 = CH_3$, X = Cl]; mp 103-104°C, in 46% yield.

^1H NMR ($CDCl_3$): ∂ 8.24 (1H, d, J = 8.0 Hz, H-4), 7.33-7.21 (3H, m, ArH), 6.35 (1H, s, $CONHCH_3$), 4.27 (2H, t, J = 7.6 Hz, $1\text{-}NC\underline{H}_2$), 3.06 (3H, d, J = 4.8 Hz, $CONHC\underline{H}_3$), 2.73 (2H, t, J = 7.5 Hz, $1\text{-}NHCH_2C\underline{H}_2$), 2.62-2.55 (4H, m, $N(C\underline{H}_2CH_3)_2$), 1.02 (6H, t, J = 7.0 Hz, $N(CH_2C\underline{H}_3)_2$).

Reaction of this with lithium methyl selenide as described above gave 2,2'-diselenobis[1-[2-(diethylamino)ethyl]-N-methyl-1H-indole-3-carboxamide] (135)

[XXIX: R_1 = H, R_2 = $CONHCH_3$, R_3 = $(CH_2)_2NEt_2$] (63% yield); mp 156-157°C.

Analysis calculated for $C_{32}H_{44}N_6O_2Se_2 \cdot 0.5H_2O$ requires:
C, 54.01; H, 6.37; N, 11.81%.
Found: C, 54.14; H, 6.23; N, 11.54%.

EXAMPLE L

Preparation of Compound 136 of Table 1 by the method outlined in Scheme 11.

An ice-cold solution of 15 g (50 mmol) of the *N*-trifluoroacetamide of D-tryptophan, synthesized by methods previously outlined (J. Org. Chem. 1979;44:2805-2807) in 50 mL of THF under N_2 was treated sequentially with 7.1 g (52.5 mmol) of 1-hydroxybenzotriazole then 10.83 g (52.5 mmol) of 1,3-dicyclohexylcarbodiimide. After 15 minutes, the solution was treated with 5.74 mL (52.6 mmol) of benzylamine. The solution was maintained at 0-5°C for 1 hour, then let warm to 25°C overnight. The mixture was filtered and the collected solids were washed with ethyl acetate. The filtrate was concentrated to an oil that was dissolved in 250 mL of ethyl acetate. The solution was washed sequentially with 250 mL portions of 10% aqueous acetic acid, water, 5% aqueous sodium hydrogen carbonate, water and brine, then dried ($NaSO_4$), and concentrated to a solid. Crystallization from 170 mL of 65:35 2-propanol:petroleum ether afforded 12.81 g (66%) of (R)-*N*-(phenylmethyl)-α-[(trifluoroacetyl)-amino]-1*H*-indole-3-propanamide [II: R_1 = H, R_2 = $CH_2CH(NHCOCF_3)CONHCH_2Ph$, R_3 = H] as an off-white solid which was used directly in the next reaction; mp 186-188°C.

To an ice-cold solution of 10 g (25.7 mmol) of (R)-*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-

1*H*-indole-3-propanamide [XXIX: R_1 = H, R_2 = $CH_2CH(NHCOCF_3)CONHCH_2Ph$, R_3 = H] in 70 mL of THF was added dropwise Se_2Cl_2. The resultant deep red suspension was stirred at 0-5°C for 4 hours, then quenched with 300 mL of water. The solids were collected by filtration, washed well with water, and air dried to leave 12 g of impure product as an orange solid. A portion of this material (10.7 g) was dissolved in 100 mL of methanol and the solution under N_2 was cooled in an ice bath. Sodium borohydride (ca 1 g) was added portionwise until there was no more color discharge. The mixture was poured immediately into a N_2 purged separatory funnel containing 200 mL of ether. The mixture was diluted with 200 mL of water, the mixture shaken, and the phases separated. The aqueous layer was treated with a small portion of additional sodium borohydride, extracted again with ether, ice-cooled, then acidified to pH 1 with concentrated HCl. The aqueous phase was extracted twice with ethyl acetate, then the combined extracts were dried ($MgSO_4$) and filtered through a pad of flash silica gel. The filtrate was concentrated to leave 5.91 g of a foam that was dissolved in ca 40 mL of absolute ethanol. The solution was kept at 25°C for several hours to initiate crystallization, then stored at 5°C. The solids were collected by filtration, washed with 2-propanol, and dried to leave 4.23 g of pure [R-(R*,R*)]-2,2'-diselenobis[*N*-(phenylmethyl)-α-[(trifluoroacetyl)amino]-1*H*-indole-3-propanamide] [XXIX: R_1 = H, R_2 = $CH_2CH(NHCOCF_3)CONHCH_2Ph$, R_3 = H], as a yellow powdery solid; mp 181-185°C.

Analysis calculated for $C_{40}H_{34}N_6O_4F_6Se_2 \cdot H_2O$ requires:
C, 50.43; H, 3.81; N, 8.82%.
Found: C, 50.47; H, 3.57; N, 8.71%.

Further processing of the filtrate by chromatography over flash SiO_2, eluting first with dichloromethane then 7% ethyl acetate in dichloromethane, provided an additional 671 mg of product following crystallization; mp 180-183°C.

A suspension of 233.5 mg (0.25 mmol) of this diselenide in 4.5 mL of dry absolute ethanol was treated with 95 mg (2.5 mmol) of sodium borohydride. The mixture was heated at reflux for 15 minutes, then treated with 95 mg of additional borohydride. The mixture was refluxed for 1.25 hours, then treated with a third 95 mg portion of borohydride. After refluxing for 30 minutes, the mixture was cooled to 25°C, diluted with methanol, and poured into an ice-cold stirring mixture of 6N HCl and ethyl acetate. The resultant mixture was stirred vigorously for 15 minutes, filtered, the phases separated, and the aqueous layer extracted once more with ethyl acetate. The combined ethyl acetate phases were then back extracted with 5% aq HCl (five times). The acidic aqueous layers were combined and diluted with an equal volume of ethyl acetate. While carefully monitoring the pH, the stirred solution was treated carefully with 10% aqueous NaOH until pH = 9.5. The resultant yellow precipitate was collected by filtration, washed well with water, and dried to leave 90 mg of [R-(R*,R*)]-2,2'-diselenobis[α-amino-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (136) [XXIX: R_1 = H, R_2 = $CH_2CH(NH_2)CONHCH_2Ph$, R_3 = H], as a yellow powder; mp 172-174°C.

^{1}H NMR ($(CD_3)_2SO$): δ 11.62 (1H, s, NH), 8.23 (1H, t, J = 5.1 Hz, N<u>H</u>CH_2), 7.61 (1H, d, J = 8.0 Hz, ArH), 7.38 (1H, d, J = 8.2 Hz, ArH), 7.35-6.95 (7H, m, ArH), 4.20, 4.17 (2x1H, 2xdd, J = 15.2, 5.8 Hz, NHC<u>H</u>$_2$), 3.46-3.40

(1H, br m, Ar-CH_2C<u>H</u>), 3.04-2.98 (1H, br m, Ar-C<u>H</u>), 2.75-2.68 (1H, br m, Ar-C<u>H</u>), 1.70 (2H, br s, NH_2). Analysis calculated for $C_{36}H_{36}N_6O_2Se_2 \cdot 1.5H_2O$ requires:

C, 56.18; H, 5.11; N, 10.68%.

Found: C, 55.91; H, 4.72; N, 10.68%.

Processing of the ethyl acetate layer from the base treatment provided 15 mg of additional product; mp 165-171°C. Total yield = 105 mg (57%).

<u>Compound 137 of Table 1</u>

Starting from the *N*-trifluroracetamide of L-tryptophan (<u>J. Org. Chem.</u> 1979;44:2805-2807) and following the same procedures as outlined for the synthesis of compound 136 of Table 1, there was obtained [S-(R*,R*)]-2,2'-diselenobis[α-amino-*N*-(phenylmethyl)-1*H*-indole-3-propanamide] (137) [XXIX: R_1 = H, R_2 = $CH_2CH(NH_2)CONHCH_2Ph$, R_3 = H] as a yellow powder; mp 171°C (dec).

BIOLOGICAL AND BIOCHEMICAL EFFECTS

<u>Tyrosine Kinase Inhibition Assay and Growth Inhibition Effects on Cells in Tissue Culture</u>

Table 2 provides representative data on inhibition of the epidermal growth factor receptor tyrosine kinase, and on cell growth inhibition.

In Table 2: No. is the compound number as recorded in Table 1.

IC50 (EGFR TK) is the concentration of drug necessary to reduce incorporation of P^{32} in GAT by 50%.

IC_{50} (PDGFR TK) is the concentration of drug necessary to reduce incorporation of P^{32} in Glu-Tyr by 50%.

IC50 growth Inhibition is (cell growth inhibition) is the concentration of drug necessary to reduce the cellular growth rate by 50%.

TABLE 2. IC_{50} Data for EGRF-R and PDGF-R Inhibition and Cell Growth Inhibition for Selected Compounds of Table 1

No.	IC_{50} (μM) or % Inhibition at 100 μM		Growth Inhibition
	EGRF-R	PDGF-R	
1	14.9	--	
2	26%	--	
3	43%	8.6%	
4	27%	--	
5	4%	--	
6	25	8.5%	
7	1.3	--	94
8	8.5	--	
9	52%	--	16
10	10%	--	34

TABLE 2 (cont'd)

No.	IC_{50} (μM) or % Inhibition at 100 μM		Growth Inhibition
	EGRF-R	PDGF-R	
11	24%	--	
12	3%	--	
13	43%	--	
14	22	--	
15	6.8	--	
16	23	--	
17	12.5%	--	
18	2%	9%	
19	10%	--	
20	9	--	
21	1.0	--	64
22	--	--	
23	--	--	
24	19%	--	
25	8.7	--	
26	23%	5%	
27	17.8	--	2.3
28	33	--	
29	8.3	--	25-100
30	9.3	--	8
31	35.5	--	1
32	34.5	4.7%	36
33	39	16.7%	3.0
34	38	12.8%	2.7
35	16.5	33.9%	
36	4.8	--	59
37	3.3	--	
38	36.5%	--	1.6
39	20.6	--	7.4
40	16.3%	--	5.2
41	8.4	--	>25
42	26%	--	
43	2.9	--	
44	16.6%	5%	2.4
45	1.6	--	
46	11.4%	--	2.7
47	0.85	--	6
48	35.5	--	

TABLE 2 (cont'd)

No.	IC_{50} (μM) or % Inhibition at 100 μM		Growth Inhibition
	EGRF-R	PDGF-R	
49	84.1	--	
50	16.0	62.6%	
51	7.0	--	
52	68.2	18.3%	
53	4.2	--	
54	29	20.6%	
55	44	--	
56	7.3	44.5%	
57	46%	14.5%	
58	68%	--	
59	30.5	11.4%	
60	53%	--	
61	37%	11%	
62	6.0	71%	5.3
63	60	--	
64	29	--	
65	17.8	--	
66	8.3	--	
67	18%	2%	
68	14%	--	1.8
69	55.6%	8.9%	
70	8.6	1%	12
71	20%	5%	52
72	47%	22%	
73	4.3	21%	9.3
74	23%	--	
75	6%	3%	4
76	7%	19%	22
77	9%	1%	
78	27%	7%	
79	11%	20%	1.9
80	0%	16%	
81	3.6	2%	17
82	6.5	--	24
83	22.3	57%	10
84	35%	22%	
85	8%	7%	
86	4.9	5%	

TABLE 2 (cont'd)

No.	IC_{50} (μM) or % Inhibition at 100 μM		Growth Inhibition
	EGRF-R	PDGF-R	
87	34%	44%	
88	54	51%	
89	11.4	3%	
90	26	36.5	
91	5.2	--	
92	--	--	
93	30%	--	
94	--	--	
95	9.4	--	
96	--	--	
97	10.1	28.1	1.8
98	1.5	9%	5-12
99	40	19%	2.8
100	18%	23%	
101	5.5	--	
102	6.1	--	
103	7%	--	3.8
104	20%	--	
105	16.9	33%	
106	34%	--	
107	12.0	--	
108	20%	--	
109	47	8%	
110	13	--	
111	5.3	76%	
112	10.0	69%	
113	5%	29%	
114	42.9	7.0	
115	26	19.7	>50
116	4%	7.9	
117	25%	4.2	
118	4.7	78%	
119	21.2	73%	
120	6.9	--	
121	5.6	--	
122	51%	--	
123	--	--	

TABLE 2 (cont'd)

No.	IC_{50} (μM) or % Inhibition at 100 μM		Growth Inhibition
	EGRF-R	PDGF-R	
125	78%	--	
126	60%	--	
127	6.8	--	
128	--	--	
129	31%	--	
130	3.5	--	
131	5.8	--	5.5
132	4.7	--	20
133	13.0	--	<5
134	4.6	--	8
135	6.9		
136			
137			

EGF Receptor Tyrosine Kinase Assay

Membrane vesicles were prepared by the method described in Cohen S, Ushiro H, Stoscheck C, and Chinkers M. A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles, J. Biol. Chem. 1982;257:1523-1531, and kept frozen at -90°C until use. At the time of assay, membranes were solubilized in 4% Triton X-100 and 10% glycerol. The reaction is carried out in wells of a 96-well microtiter plate in a total volume of 125 L. Buffer containing 20 mM Hepes (pH 7.4), 15 mM $MgCl_2$, 4 mM $MnCl_2$, and 0.02% BSA followed by 5 to 20 mg of membrane protein and 150 ng of epidermal growth factor. The plates are incubated for 10 minutes at room temperature to activate the receptor kinase. 20 g of GAT (random polymer of glycine, alanine, and tyrosine) and 0.2 mCi of α-[P^{32}] ATP plus or minus

compound are added and incubated 10 minutes at room temperature. The reaction is stopped by addition of 125 mL of 30% TCA, precipitate washed twice with 200 mL of 15% TCA on 0.65 micron filters, and the filters counted by scintillation spectrometry.

PDGF Receptor Tyrosine Kinase Inhibition Assay

Recombinant baculovirus containing human PDGF β receptor intracellular tyrosine kinase domain was used to infect SF9 cells to overexpress the protein, and cell lysates were used for the assay. The ability of the tyrosine kinase to phosphorylate glutamate - tyrosine substrate in the presence of P^{32}-ATP and inhibitor was measured by counting the incorporation of P^{32} in Glu-Tyr in TCA precipitable material.

Table 2 provides representative data on inhibition of the PDGF receptor tyrosine kinase. In Table 2, No. refers to the compound number as recorded in Table 1.

DETAILED STUDIES ON THE BIOLOGICAL EFFECTS OF COMPOUNDS 21 AND 70

Effects on Cells in Tissue Culture

Swiss 3T3 fibroblasts, that were growth arrested in serum-free media for 24 hours, were exposed to various concentrations of compound for 2 hours. The cells were then exposed to individual growth factors for 5 minutes and proteins that were phosphorylated on tyrosine in response to the mitogens and were detected by Western blotting techniques using phosphotryosine antibodies. Similar techniques were used for tumor cell lines except the time in serum-free media was increased.

At concentrations of 10 to 50 mM, Compound 21 suppressed: (1) EGF mediated phosphorylation of a variety of endogenous proteins; (2) PDGF mediated autophosphorylation of the PDGF receptor as well as PDGF mediated tyrosine phosphorylation of other endogenous proteins and; (3) bFGF mediated tyrosine phosphorylation. 70 was more selective and inhibited only bFGF mediated tyrosine phosphorylation and at concentrations as low as 2 mM.

Effects on Growth Factor Mediated Mitogenesis

Swiss 3T3 fibroblasts, that were growth arrested in serum-free media for 24 hours, were exposed to various concentrations of compound for 2 hours. The cells were then exposed to individual growth factors for 24 hours and mitogenesis assessed by measuring tritiated thymidine incorporation into DNA.

The concentration of 21 and 70 required to inhibit growth factor mediated mitogenesis by 50% for the following growth factors was as follows:

Growth Factor	IC_{50} (μM) for 21	IC_{50} (μM) for 70
EGF	2	3
PDGF	8	4
bFGF	13	3
serum	19	3

Growth Inhibition Assay

Swiss 3T3 mouse fibroblasts were maintained in dMEM/F12 media containing 10% fetal calf serum. Two mL of cells at a density of 1 x 10^4/mL were placed in 24-well plates plus or minus various concentrations of

the inhibitor. The cells were grown at 37°C under 5% CO_2 for 72 hours and then counted by Coulter counter. The data were expressed as the concentration of inhibitor necessary to decrease the growth rate by 50%.

Compound 21 was growth inhibitory for a variety of human tumor cell lines as well as the Swiss 3T3 fibroblasts. The concentration of 21 necessary to inhibit cell growth by 50% is shown below:

Cell Line	IC_{50} (μM)
MDA 468 breast	43
A431 epidermoid	62
A549 lung	30
MDV-7 breast	39
MDA-231 breast	15
Swiss 3T3 fibroblasts	64
HT-29 colon	55

Although the carboxyl containing structures are among the most active enzyme inhibitors, they are poorly transported into the cell, whereas the less active esters are transported efficiently and once in the cytoplasm rendered highly active by esterases. Esters may, therefore, be more favorable than carboxylic acids in this invention.

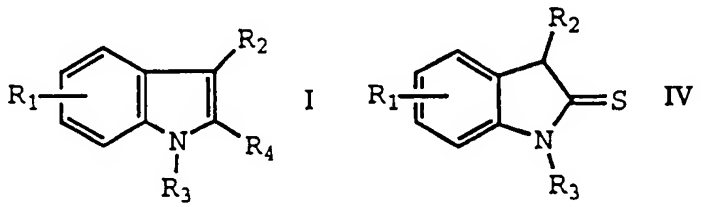
The data of Table 2 show that the 2-thioindoles of general Formula I listed in Table 1 include compounds which are active as potent inhibitors of protein tyrosine kinases and as cytotoxic agents.

The invention is not limited to the particular embodiments shown and described herein, since various changes and modifications may be made without departing

from the spirit and scope of the invention as defined by the following claims.

CLAIMS

1. 2-Thioindole, 2-indolinethione and polysulfide compounds of the group represented by the general Formulas I and IV



and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OCOR, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and further represents replacement in the ring of 1 or 2 ring methine (-CH=) atoms with aza(-N=) atoms;

R_2 is a member selected from

C_{2-4} alkyl,

$(CH_2)_nCOOH$,

$(CH_2)_nCOOR$,

$(CH_2)_nCOR$,

$(CH_2)_nSO_2R$,

$(CH_2)_nSO_2NRR$,

$(CH_2)_nSO_2NHR$,

CH=CHCOOH,

$(CH_2)_nCH(OH)\text{-}COOH$,

$(CH_2)_nCH(NH_2)\text{-}COOH$,

$(CH_2)_nCONH_2$,

$(CH_2)_nCONRR$,
$(CH_2)_nCONHCH_2Ph$,
CONHR,
CONRR,
CONHPh,
COY,
COPhCOOH,
COPhCOOR,
$(CH_2)_nCONHPh$,
$(CH_2)_nCONHPhR$,
SO_2Y;

n is an integer from 1 to 4;

R is lower alkyl;

R_3 is a member selected from H, lower alkyl, and benzyl;

Y represents a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

R_4 represents SH, S_oX, and S_oQ where o is 1, 2, or 3, X is a member selected from H, lower alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-thioindolyl moiety of Formula I provided that the group does not comprise compounds having the names

2-(2-thioxo-3-indolinyl)acetic acid,
2-(1-methyl-2-thioxo-3-indolinyl)acetic acid,
methyl 2-(2-thioxo-3-indolinyl)acetate,
ethyl 2-(1-methyl-2-thioxo-3-indolinyl)-acetate,
bis[methylindolinyl-3-acetate-(2)]disulfide,
bis[indolyl-3-acetic acid-(2)]disulfide,

bis[methylindolyl-3-acetate-(2)]trisulfide, and
bis[1-methylindolyl-3-acetic acid-(2)]-disulfide.

2. A thioindole compound according to Claim 1 selected from
methyl 2-(1-methyl-2-thioxo-3-indolinyl)-acetate,
N-benzyl(2-thioxo-3-indolinyl)acetamide,
3-(2-thioxo-3-indolinyl)propanoic acid,
3-(1-methyl-2-thioxo-3-indolinyl)propanoic acid,
methyl 3-(2-thioxo-3-indolinyl)propanoate,
ethyl 3-(2-thioxo-3-indolinyl)propanoate,
3-(1-methyl-2-thioxo-3-indolinyl)propanoate,
ethyl 3-(1-methyl-2-thioxo-3-indolinyl)-propanoate,
N-benzyl 3-(2-thioxo-3-indolinyl)propanamide,
4-(2-thioxo-3-indolinyl)butanoic acid,
4-(1-methyl-2-thioxo-3-indolinyl)butanoic acid,
methyl 4-(2-thioxo-3-indolinyl)butanoate,
methyl 4-(1-methyl-2-thioxo-3-indolinyl)-butanoate,
N-phenyl (1-methyl-2-thioxo-3-indolinyl)-carboxamide,
N-phenyl (1-methyl-2-methylthio-3-indolinyl) carboxamide,
3-benzoyl-1-methyl-2-indolinethione,
3-(4'-carboxybenzoyl)-1-methyl-2-indolinethione,

3-(4'-carbomethoxybenzoyl)-1-methyl-2-indolinethione,

and pharmaceutically acceptable salts thereof.

3. A polysulfide compound according to Claim 1 selected from

2,2'-dithiobis[methyl 2-(1-methyl-3-indolyl)acetate],

bis[indolyl-3-acetic acid-(2)]trisulfide,

bis[ethyl 1-methylindolyl-3-acetate-(2)]-disulfide,

2,2'-dithiobis[N-benzyl-2-(3-indolyl)-acetamide],

bis[indolyl-3-propanoic acid-(2)]disulfide,

2,2'-dithiobis[3-(1-methyl-3-indolyl)-propanoic acid],

bis[ethylindolyl-3-propanoate-(2)]disulfide,

2,2'-dithiobis[methyl-3-(3-indolyl)-propanoate],

2,2'-dithiobis[methyl-3-(1-methyl-3-indolyl)-propanoate],

bis[5-methylindolyl-3-propanoic acid-(2)]-disulfide,

bis[ethyl-5-methylindolyl-3-propanoate-(2)]-disulfide,

bis[6-methylindolyl-3-propanoic acid-(2)]-disulfide,

bis[ethyl-6-methylindolyl-3-propanoate-(2)]-disulfide,

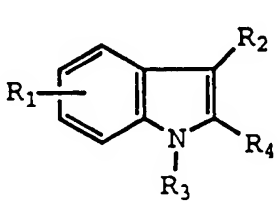
bis[7-methylindolyl-3-propanoic acid-(2)]-disulfide,

bis[ethyl-7-methylindolyl-3-propanoate-(2)]-disulfide,

2,2'-dithiobis[N-benzyl-3-(3-indolyl)-propanamide],
bis[indolyl-3-butanoic acid-(2)]disulfide,
2,2'-dithiobis[4-(1-methyl-3-indolyl butanoic acid],
bis[methyl indolyl-3-butanoate-(2)]disulfide,
bis[methyl 1-methylindolyl-3-butanoate-(2)]-disulfide,
bis[N-phenyl 1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 1-ethylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 4-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 5-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 7-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 1-methyl-7-azaindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 1,4-dimethylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 1,5-dimethylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 1,6-dimethylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 1,7-dimethylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 4-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 5-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-phenyl 6-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide,

bis[N-phenyl 7-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-methyl 1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-benzyl 1-methylindolyl-3-carboxamide-(2)]disulfide,
bis[N-methylphenylsulfonyl)-2-indolyl]-disulfide,
bis[3-benzoyl-1-methylindole-(2)]disulfide,
bis[3-(4'-carboxybenzoyl)-1-methylindole-(2)]-disulfide,
bis[3-(4'-carbomethoxybenzoyl)-1-methylindole(2)]disulfide,
and pharmaceutically acceptable salts thereof.

4. A pharmaceutical composition useful for inhibition of protein tyrosine kinase dependent disease in a mammal, containing in a pharmaceutically acceptable carrier a therapeutically effective amount of a compound selected from 2-thioindole, 2-indolinethione, and polysulfide compounds represented by the general Formulas I and IV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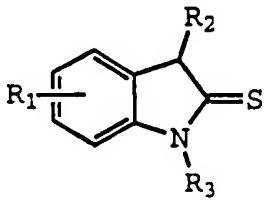

IV

and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and

further represents replacement in the ring of 1 or 2 ring methine (-CH=) atoms with aza(-N=) atoms;

R_2 is a member selected from

C_{2-4} alkyl,
$(CH_2)_nCOOH$,
$(CH_2)_nCOOR$,
$(CH_2)_nCOR$,
$(CH_2)_nSO_2R$,
$(CH_2)_nSO_2NRR$,
$(CH_2)_nSO_2NHR$,
$CH=CHCOOH$,
$(CH_2)_nCH(OH)-COOH$,
$(CH_2)_nCH(NH_2)-COOH$,
$(CH_2)_nCONH_2$,
$(CH_2)_nCONHR$,
$(CH_2)_nCONRR$,
$(CH_2)_nCONHCH_2Ph$,
CONHR,
CONRR,
CONHPh,
COY,
COPhCOOH,
COPhCOOR,
$(CH_2)_nCONHPh$,
$(CH_2)_nCONHPhR$,
SO_2Y;

n is an integer from 1 to 4;

R is lower alkyl;

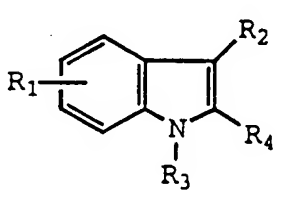
R_3 is a member selected from H, lower alkyl, and benzyl;

Y represents a benzene, pyridine, thiophene,

substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

R_4 represents SH, S_oX, and S_oQ where o is 1, 2, or 3, X is a member selected from H, lower alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-thioindolyl moiety of Formula I.

5. A pharmaceutical composition useful for treating aberrant cell growth in a mammal containing in a pharmaceutically acceptable carrier a therapeutically effective amount of a compound selected from 2-thioindole, 2-indolinethione, and polysulfide compounds represented by the general Formulas I and IV



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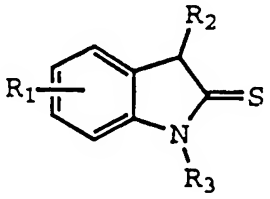


IV

and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and further represents replacement in the ring of 1 or 2 ring methine (-CH=) atoms with aza(-N=) atoms;

R_2 is a member selected from

C_{2-4} alkyl,

$(CH_2)_nCOOH$,

$(CH_2)_nCOOR$,

$(CH_2)_nCOR$,

$(CH_2)_nSO_2R$,

$(CH_2)_nSO_2NRR$,
$(CH_2)_nSO_2NHR$,
CH=CHCOOH,
$(CH_2)_nCH(OH)COOH$,
$(CH_2)_nCH(NH_2)COOH$,
$(CH_2)_nCONH_2$,
$(CH_2)_nCONHR$,
$(CH_2)_nCONRR$,
$(CH_2)_nCONHCH_2Ph$,
CONHR,
CONHPh,
COY,
COPhCOOH,
COPhCOOR,
$(CH_2)_nCONHPh$,
$(CH_2)_nCONHPhR$,
SO_2Y;

n is an integer from 1 to 4;

R is lower alkyl;

R_3 is a member selected from H, lower alkyl, and benzyl;

Y represents a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

R_4 represents SH, S_oX, and S_oQ where o is 1, 2, or 3, X is a member selected from H, lower alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-thioindolyl moiety of Formula I.

6. The compound of Claim 1 having the name 3-(2-thioxo-3-indolinyl)propanoic acid.

7. The compound of Claim 1 having the name 4-(2-thioxo-3-indolinyl)butanoic acid and pharmaceutically acceptable salts thereof.

8. The compound of Claim 1 having the name benzyl[N-phenyl 1-methylindolyl-3-carboxamide(2)]-disulfide.

9. The compound of Claim 1 having the name bis[indolyl-3-acetic acid-(2)]trisulfide.

10. The compound of Claim 1 having the name N-benzyl(2-thioxo-3-indolinyl)acetamide and pharmaceutically acceptable salts thereof.

11. The compound of Claim 1 having the name bis[indolyl-3-propanoic acid-(2)]disulfide and pharmaceutically acceptable salts thereof.

12. The compound of Claim 1 having the name 2,2'-dithiobis[3-(1-methyl-3-indolyl)propanoic acid] and pharmaceutically acceptable salts thereof.

13. The compound of Claim 1 having the name bis[ethylindolyl-3-propanoate-(2)]disulfide.

14. The compound of Claim 1 having the name 2,2'-dithiobis[methyl-3-(1-methyl-3-indolyl)-propanoate].

15. The compound of Claim 1 having the name bis[6-methylindolyl-3-propanoic acid-(2)]disulfide and pharmaceutically acceptable salts thereof.

16. The compound of Claim 1 having the name bis[ethyl-6-methylindolyl-3-propanoate(2)]-disulfide.

17. The compound of Claim 1 having the name bis[7-methylindolyl-3-propanoic acid-(2)]disulfide and pharmaceutically acceptable salts thereof.

18. The compound of Claim 1 having the name 2,2'-dithiobis[N-benzyl-3-(3-indolyl)propanamide].

19. The compound of Claim 1 having the name 2,2'-dithiobis[4-(1-methyl-3-indolyl)butanoic acid] and pharmaceutically acceptable salts thereof.

20. The compound of Claim 1 having the name bis[methyl 1-methylindolyl-3-butanoate-(2)]disulfide.

21. The compound of Claim 1 having the name bis[N-phenyl 1-methylindolyl-3-carboxamide(2)]-disulfide.

22. The compound of Claim 1 having the name bis[N-phenyl 5-chloro-1-methylindolyl-3-carboxamide-(2)]disulfide.

23. The compound of Claim 1 having the name bis[N-phenyl 6-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide.

24. The compound of Claim 1 having the name bis[N-phenyl 7-methoxy-1-methylindolyl-3-carboxamide-(2)]disulfide.

25. The compound of Claim 1 having the name bis[N-methyl 1-methylindolyl-3-carboxamide(2)]-disulfide.

26. The compound of Claim 1 having the name bis[N-benzyl 1-methylindolyl-3-carboxamide(2)]-disulfide.

27. The compound of Claim 1 having the name bis[N-methylphenylsulfonyl)-2-indolyl]disulfide.

28. The compound of Claim 1 having the name bis[3-(4'-carboxybenzoyl)-1-methylindole-(2)]disulfide.

29. The compound of Claim 1 having the name bis[3-(4'-carbomethoxybenzoyl)-1-methylindole-(2)]-disulfide.

30. The compound of Claim 1 having the name methyl 3-(1-methyl-2-thioxo-3-indolinyl)propanoate.

31. The compound of Claim 1 having the name ethyl 3-(1-methyl-2-thioxo-3-indolinyl)propanoate.

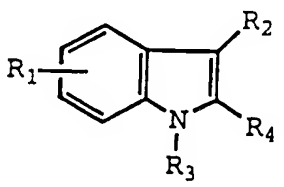
32. The compound of Claim 1 having the name N-benzyl 3-(2-thioxo-3-indolinyl)propanamide.

33. A method for inhibiting protein tyrosine kinase dependent disease in a mammal, comprising

administering to said mammal a pharmaceutical composition according to Claim 4.

34. A method for treating aberrant cell growth in a mammal, comprising administering to said mammal a pharmaceutical composition according to Claim 5.

35. 2-Selenoindole, 2-indolineselenone and selenide compounds of the group represented by the general Formulas I and XXXII



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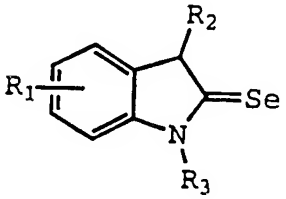


XXXII

and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OCOR, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and further represents replacement in the ring of 1 or 2 ring methine (-CH=) atoms with aza(-N=) atoms;

R_2 is a member selected from

C_{2-4} alkyl,
$(CH_2)_nCOOH$,
$(CH_2)_nCOOR$,
$(CH_2)_nCOR$,
$(CH_2)_nSO_2R$,
$(CH_2)_nSO_2NRR$,
$(CH_2)_nSO_2NHR$,
CH=CHCOOH,

$(CH_2)_nCH(OH)\text{-}COOH$,

$(CH_2)_nCH(NH_2)\text{-}COOH$,

$(CH_2)_nCONH_2$,

$(CH_2)_nCONHR$,

$(CH_2)_nCONRR$,

$(CH_2)_nCONHCH_2Ph$,

CONHR,

CONRR,

CONHPh,

COY,

COPhCOOH,

COPhCOOR,

$(CH_2)_nCONHPh$,

$(CH_2)_nCONHPhR$,

SO_2Y;

n is an integer from 1 to 4;

R is lower alkyl;

R_3 is a member selected from H, lower alkyl, and benzyl;

Y represents a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

R_4 represents SeH, Se_oX, and Se_oQ where o is 1, 2, or 3, X is a member selected from H, lower alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-selenoindolyl moiety of Formula I.

36. A selenide compound according to Claim 35 selected from

2,2'-diselenobis[1-methyl-1H-indole-3-carboxylic acid, t-butyl ester],

2,2'-diselenobis[1-methyl-1H-indole-3-carboxylic acid],

2,2'-diselenobis[N,1-dimethyl-1H-indole-3-carboxamide],

2,2'-diselenobis[N-[2-(diethylamino)ethyl]-1-methyl-1H-indole-3-carboxamide],

2,2'-diselenobis[N-methyl-1H-indole-3-carboxamide],

2,2'-diselenobis[N-[2-(diethylamino)ethyl]-1H-indole-3-carboxamide],

2,2'-diselenobis[N-[2-(diethylamino)ethyl]-N-methyl-1H-indole-3-carboxamide],

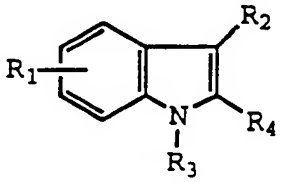
2,2'-diselenobis[1-[2-(diethylamino)ethyl]-N-methyl-1H-indole-3-carboxamide],

[R-(R*,R*)]-2,2'-diselenobis[α-amino-N-(phenylmethyl)-1H-indole-3-propanamide], or

[S-(R*,R*)]-2,2'-diselenobis[α-amino-N-(phenylmethyl)-1H-indole-3-propanamide]

and pharmaceutically acceptable salts thereof.

37. A pharmaceutical composition useful for inhibition of protein tyrosine kinase dependent disease in a mammal, containing in a pharmaceutically acceptable carrier a therapeutically effective amount of a compound selected from 2-selenoindole, 2-indolineselenone, and selenide compounds represented by the general Formulas I and XXXII



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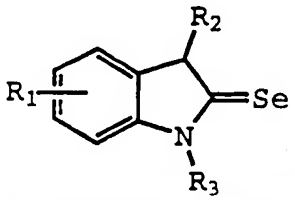


XXXII

and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and further represents replacement in the ring of 1 or 2 ring methine (-CH=) atoms with aza(-N=) atoms;

R_2 is a member selected from

C_{2-4} alkyl,
$(CH_2)_nCOOH$,
$(CH_2)_nCOOR$,
$(CH_2)_nCOR$,
$(CH_2)_nSO_2R$,
$(CH_2)_nSO_2NRR$,
$(CH_2)_nSO_2NHR$,
CH=CHCOOH,
$(CH_2)_nCH(OH)\text{-}COOH$,
$(CH_2)_nCH(NH_2)\text{-}COOH$,
$(CH_2)_nCONH_2$,
$(CH_2)_nCONHR$,
$(CH_2)_nCONRR$,
$(CH_2)_nCONHCH_2Ph$,
CONHR,
CONRR,
CONHPh,
COY,
COPhCOOH,
COPhCOOR,
$(CH_2)_nCONHPh$,
$(CH_2)_nCONHPhR$,
SO_2Y;

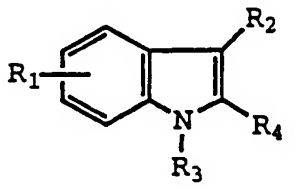
R is lower alkyl;

R_3 is a member selected from H, lower alkyl, and benzyl;

Y represents a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

R_4 represents SeH, Se_oX, and Se_oQ where o is 1, 2, or 3, X is a member selected from H, lower alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-selenoindolyl moiety of Formula I.

38. A pharmaceutical composition useful for treating aberrant cell growth in a mammal containing in a pharmaceutically acceptable carrier a therapeutically effective amount of a compound selected from 2-selenoindole, 2-indolineselenone, and selenide compounds represented by the general Formulas I and XXXII



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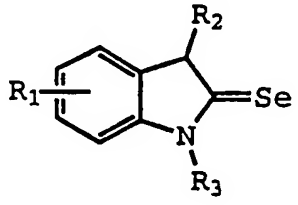

XXXII

and pharmaceutically acceptable salts thereof, wherein

R_1 is a member selected from H, halogen, R, OH, OR, CF_3, NO_2, NH_2, NHR, COOH, CONHR, $(CH_2)_nOH$, $(CH_2)_nOR$, $(CH_2)_nNH_2$, $(CH_2)_nNHR$, and $(CH_2)_nNRR$, and further represents replacement in the ring of 1 or 2 ring methine (-CH=) atoms with aza(-N=) atoms;

R_2 is a member selected from

C_{2-4} alkyl,
$(CH_2)_nCOOH$,
$(CH_2)_nCOOR$,
$(CH_2)_nCOR$,
$(CH_2)_nSO_2R$,
$(CH_2)_nSO_2NRR$,
$(CH_2)_nSO_2NHR$,
CH=CHCOOH,
$(CH_2)_nCH(OH)\text{-}COOH$,
$(CH_2)_nCH(NH_2)\text{-}COOH$,
$(CH_2)_nCONH_2$,
$(CH_2)_nCONHR$,
$(CH_2)_nCONRR$,
$(CH_2)_nCONHCH_2Ph$,
CONHR,
CONHPh,
COY,
COPhCOOH,
COPhCOOR,
$(CH_2)_nCONHPh$,
$(CH_2)_nCONHPhR$,
SO_2Y;

n is an integer from 1 to 4;

R is lower alkyl;

R_3 is a member selected from H, lower alkyl, and benzyl;

Y represents a benzene, pyridine, thiophene, furan, thiazole, or imidazole ring optionally substituted with a lower alkyl, COOH, OH, OCOR, NH_2, CONHR, CONRR, OR, or NHR group; and

R_4 represents SeH, Se_oX, and Se_oQ where o is 1, 2, or 3. X is a member selected from H

alkyl, benzyl, and benzene, pyridine, thiophene, furan, thiazole, and imidazole rings, and Q is another 2-selenoindolyl moiety of Formula I.

39. The compound of Claim 35 having the name [R-(R*,R*)]-2,2'-diselenobis[α-amino-N-(phenylmethyl)-1H-indole-3-propanamide].

40. The compound of Claim 35 having the name [S-(R*,R*)]-2,2'-diselenobis[α-amino-N-(phenylmethyl)-1H-indole-3-propanamide].

41. The compound of Claim 35 having the name 2,2'-diselenobis[1-methyl-1H-indole-3-carboxylic acid, t-butyl ester].

42. The compound of Claim 35 having the name 2,2'-diselenobis[1-methyl-1H-indole-3-carboxylic acid].

43. The compound of Claim 35 having the name 2,2'-diselenobis[N,1-dimethyl-1H-indole-3-carboxamide].

44. The compound of Claim 35 having the name 2,2'-diselenobis[N-[2-(diethylamino)ethyl]-1-methyl-1H-indole-3-carboxamide].

45. The compound of Claim 35 having the name 2,2'-diselenobis[N-1-methyl-1H-indole-3-carboxamide].

46. The compound of Claim 35 having the name 2,2'-diselenobis[N-[2-(diethylamino)ethyl]-1H-indole-3-carboxamide].

47. The compound of Claim 35 having the name 2,2'-diselenobis[N-[2-(diethylamino)ethyl]-N-methyl-1H-indole-3-carboxamide].

48. The compound of Claim 35 having the name 2,2'-diselenobis[1-[2-(diethylamino)ethyl]-N-methyl-1H-indole-3-carboxamide].

49. A method for inhibiting protein tyrosine kinase dependent disease in a mammal comprising administering to said mammal a pharmaceutical composition according to Claim 37.

50. A method for treating aberrant cell growth in a mammal, comprising administering to said mammal a pharmaceutical composition according to Claim 38.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/07272

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D209/30; C07D209/42; C07D405/14; C07D409/14
C07D401/14; C07D471/04; A61K31/40; A61K31/44

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	BULLETIN DE LA SOCIETE CHIMIQUE DE FRANCE vol. 1, 1987, pages 181 - 188 'A new preparation of N-aryl-1-alkyynesulphenamides and their thermal rearrangements into indoline-2-thiones' *see compounds of examples 29,30 and 32* ---	1
X	TETRAHEDRON vol. 42, 1986, pages 5879 - 5886 'Synthesis of debromo-8,8a-dihydroflustramine C, a model synthesis towards amauromine' cited in the application *see compound number 12, page 5881* --- -/--	1

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 NOVEMBER 1993	- 9. 12. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	SCRUTON-EVANS I.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	TETRAHEDRON LETTERS vol. 31, 1990, pages 7229 - 7232 'Selectivity in the Thiocyanation of 3.alkylindoles: An unexpectedly easy access to 2-isothiocyano derivatives' cited in the application	1-32
T	JOURNAL OF MEDICINAL CHEMISTRY vol. 36, 1993, pages 2459 - 2469 'Tyrosine kinase inhibitors'	1-32, 35-48
A	WO,A,9 113 055 (FARMITALIA CARLO ERBA S.R.L) 5 September 1991	1-32, 35-48
P,A	US,A,5 196 446 (YISSUM RESEARCH DEVELOPMENT CO. OF THE HEBREW UNIVERSITY OF JERUSALEM) 23 March 1993	1-32

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9307272
SA 78016

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9113055	05-09-91	AU-A-	7241291	18-09-91
		EP-A-	0470221	12-02-92
		JP-T-	4506081	22-10-92
US-A-5196446	23-03-93	AU-A-	7756891	11-11-91
		EP-A-	0527181	17-02-93
		WO-A-	9116305	31-10-91

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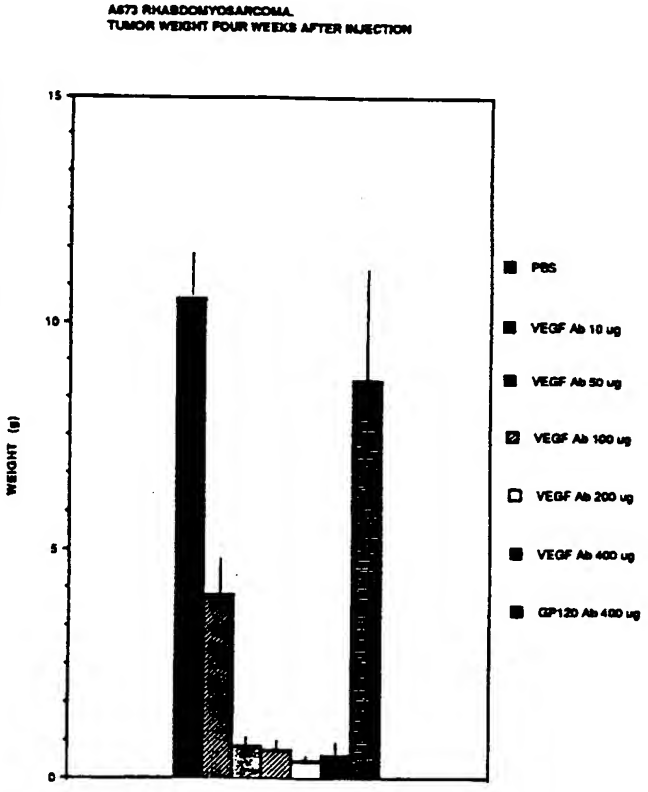
(54) Title: VASCULAR ENDOTHELIAL CELL GROWTH FACTOR ANTAGONISTS

(57) Abstract

The present invention provides vascular endothelial cell growth factor (hVEGF) antagonists, including monoclonal antibodies, hVEGF receptors, and hVEGF variants that inhibit the mitogenic, angiogenic, or other biological activity of hVEGF. The antagonists thus are useful for the treatment of diseases and disorders characterized by undesirable or excessive endothelial cell proliferation or neovascularization. The monoclonal antibodies and receptors of the invention are also useful in diagnostic and analytical methods for determining the presence of hVEGF in a test sample.



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VASCULAR ENDOTHELIAL CELL GROWTH FACTOR ANTAGONISTS

Field of the Invention

The present invention relates to vascular endothelial cell growth factor (VEGF) antagonists, to therapeutic compositions comprising the antagonists, and to methods of use of the antagonists for diagnostic and therapeutic purposes.

Background of the Invention

The two major cellular components of the vasculature are the endothelial and smooth muscle cells. The endothelial cells form the lining of the inner surface of all blood vessels, and constitute a nonthrombogenic interface between blood and tissue. In addition, endothelial cells are an important component for the development of new capillaries and blood vessels. Thus, endothelial cells proliferate during the angiogenesis, or neovascularization, associated with tumor growth and metastasis, and a variety of non-neoplastic diseases or disorders.

Various naturally occurring polypeptides reportedly induce the proliferation of endothelial cells. Among those polypeptides are the basic and acidic fibroblast growth factors (FGF), Burgess and Maciag, Annual Rev. Biochem., 58:575 (1989), platelet-derived endothelial cell growth factor (PD-ECGF), Ishikawa, et al., Nature, 338:557 (1989), and vascular endothelial growth factor (VEGF), Leung, et al., Science 246:1306 (1989); Ferrara & Henzel, Biochem. Biophys. Res. Commun. 161:851 (1989); Tischer, et al., Biochem. Biophys. Res. Commun. 165:1198 (1989); Ferrara, et al., PCT Pat. Pub. No. WO 90/13649 (published November 15, 1990); Ferrara, et al., U.S. Pat. App. No. 07/360,229.

VEGF was first identified in media conditioned by bovine pituitary follicular or folliculostellate cells. Biochemical analyses indicate that bovine VEGF is a dimeric protein with an apparent molecular mass of approximately 45,000 Daltons, and with an apparent mitogenic specificity for vascular endothelial cells. DNA encoding bovine VEGF was isolated by screening a cDNA library prepared from such cells, using oligonucleotides based on the amino-terminal amino acid sequence of the protein as hybridization probes.

Human VEGF was obtained by first screening a cDNA library prepared from human cells, using bovine VEGF cDNA as a hybridization probe. One cDNA identified thereby encodes a 165-amino acid protein having greater than 95% homology to bovine VEGF, which protein is referred to as human VEGF (hVEGF). The mitogenic activity of human VEGF was confirmed by expressing the human VEGF cDNA in mammalian host cells. Media conditioned by cells transfected with the human VEGF cDNA promoted the proliferation of capillary endothelial cells, whereas control cells did not. Leung, et al., Science 246:1306 (1989).

Several additional cDNAs were identified in human cDNA libraries that encode 121-, 189-, and 206-amino acid isoforms of hVEGF (also collectively referred to as hVEGF-related proteins). The 121-amino acid protein differs from hVEGF by virtue of the deletion of the 44 amino acids between residues 116 and 159 in hVEGF. The 189-amino acid protein differs

from hVEGF by virtue of the insertion of 24 amino acids at residue 116 in hVEGF, and apparently is identical to human vascular permeability factor (hVPF). The 206-amino acid protein differs from hVEGF by virtue of an insertion of 41 amino acids at residue 116 in hVEGF. Houck, et al., Mol. Endocrin. 5:1806 (1991); Ferrara, et al., J. Cell. Biochem. 47:211 (1991); Ferrara, et al., Endocrine Reviews 13:18 (1992); Keck, et al., Science 246:1309 (1989); Connolly, et al., J. Biol. Chem. 264:20017 (1989); Keck, et al., EPO Pat. Pub. No. 0 370 989 (published May 30, 1990).

VEGF not only stimulates vascular endothelial cell proliferation, but also induces vascular permeability and angiogenesis. Angiogenesis, which involves the formation of new blood vessels from preexisting endothelium, is an important component of a variety of diseases and disorders including tumor growth and metastasis, rheumatoid arthritis, psoriasis, atherosclerosis, diabetic retinopathy, retrolental fibroplasia, neovascular glaucoma, hemangiomas, immune rejection of transplanted corneal tissue and other tissues, and chronic inflammation.

In the case of tumor growth, angiogenesis appears to be crucial for the transition from hyperplasia to neoplasia, and for providing nourishment to the growing solid tumor. Folkman, et al., Nature 339:58 (1989). Angiogenesis also allows tumors to be in contact with the vascular bed of the host, which may provide a route for metastasis of the tumor cells. Evidence for the role of angiogenesis in tumor metastasis is provided, for example, by studies showing a correlation between the number and density of microvessels in histologic sections of invasive human breast carcinoma and actual presence of distant metastases. Weidner, et al., New Engl. J. Med. 324:1 (1991).

In view of the role of vascular endothelial cell growth and angiogenesis, and the role of those processes in many diseases and disorders, it is desirable to have a means of reducing or inhibiting one or more of the biological effects of VEGF. It is also desirable to have a means of assaying for the presence of VEGF in normal and pathological conditions, and especially cancer.

Summary of the Invention

The present invention provides antagonists of VEGF, including (a) antibodies and variants thereof which are capable of specifically binding to hVEGF, hVEGF receptor, or a complex comprising hVEGF in association with hVEGF receptor, (b) hVEGF receptor and variants thereof, and (c) hVEGF variants. The antagonists inhibit the mitogenic, angiogenic, or other biological activity of hVEGF, and thus are useful for the treatment of diseases or disorders characterized by undesirable excessive neovascularization, including by way of example tumors, and especially solid malignant tumors, rheumatoid arthritis, psoriasis, atherosclerosis, diabetic and other retinopathies, retrolental fibroplasia, neovascular glaucoma, hemangiomas, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, and chronic inflammation. The antagonists also are useful for the treatment

of diseases or disorders characterized by undesirable excessive vascular permeability, such as edema associated with brain tumors, ascites associated with malignancies, Meigs' syndrome, lung inflammation, nephrotic syndrome, pericardial effusion (such as that associated with pericarditis), and pleural effusion.

In other aspects, the VEGF antagonists are polyspecific monoclonal antibodies which are capable of binding to (a) a non-hVEGF epitope, for example, an epitope of a protein involved in thrombogenesis or thrombolysis, or a tumor cell surface antigen, and to (b) hVEGF, hVEGF receptor, or a complex comprising hVEGF in association with hVEGF receptor.

In still other aspects, the VEGF antagonists are conjugated with a cytotoxic moiety.

In another aspect, the invention concerns isolated nucleic acids encoding the monoclonal antibodies as hereinbefore described, and hybridoma cell lines which produce such monoclonal antibodies.

In another aspect, the invention concerns pharmaceutical compositions comprising a VEGF antagonist in an amount effective in reducing or eliminating hVEGF-mediated mitogenic or angiogenic activity in a mammal.

In a different aspect, the invention concerns methods of treatment comprising administering to a mammal, preferably a human patient in need of such treatment, a physiologically effective amount of a VEGF antagonist. If desired, the VEGF antagonist is coadministered, either simultaneously or sequentially, with one or more other VEGF antagonists or anti-tumor or anti-angiogenic substances.

In another aspect, the invention concerns a method for detecting hVEGF in a test sample by means of contacting the test sample with an antibody capable of binding specifically to hVEGF and determining the extent of such binding.

Brief Description of the Drawings

Figure 1 shows the effect of anti-hVEGF monoclonal antibodies (A4.6.1 or B2.6.2) or an irrelevant anti-hepatocyte growth factor antibody (anti-HGF) on the binding of the anti-hVEGF monoclonal antibodies to hVEGF.

Figure 2 shows the effect of anti-hVEGF monoclonal antibodies (A4.6.1 or B2.6.2) or an irrelevant anti-HGF antibody on the biological activity of hVEGF in cultures of bovine adrenal cortex capillary endothelial (ACE) cells.

Figure 3 shows the effect of anti-hVEGF monoclonal antibodies (A4.6.1, B2.6.2, or A2.6.1) on the binding of hVEGF to bovine ACE cells.

Figure 4 shows the effect of A4.6.1 anti-hVEGF monoclonal antibody treatment on the rate of growth of growth of NEG55 tumors in mice.

Figure 5 shows the effect of A4.6.1 anti-hVEGF monoclonal antibody treatment on the size of NEG55 tumors in mice after five weeks of treatment.

Figure 6 shows the effect of A4.6.1 anti-hVEGF monoclonal antibody (VEGF Ab) treatment on the growth of SK-LMS-1 tumors in mice.

Figure 7 shows the effect of varying doses of A4.6.1 anti-hVEGF monoclonal antibody (VEGF Ab) treatment on the growth of A673 tumors in mice. is shown in

Figure 8 shows the effect of A4.6.1 anti-hVEGF monoclonal antibody on the growth and survival of NEG55 (G55) glioblastoma cells in culture.

Figure 9 shows the effect of A4.6.1 anti-hVEGF monoclonal antibody on the growth and survival of A673 rhabdomyosarcoma cells in culture.

Figure 10 shows the effect of A4.6.1 anti-hVEGF monoclonal antibody on human synovial fluid-induced chemotaxis of human endothelial cells.

Detailed Description of the Invention

The term "hVEGF" as used herein refers to the 165-amino acid human vascular endothelial cell growth factor, and related 121-, 189-, and 206-amino acid vascular endothelial cell growth factors, as described by Leung, et al., Science 246:1306 (1989), and Houck, et al., Mol. Endocrin. 5:1806 (1991) together with the naturally occurring allelic and processed forms of those growth factors.

The present invention provides antagonists of hVEGF which are capable of inhibiting one or more of the biological activities of hVEGF, for example, its mitogenic or angiogenic activity. Antagonists of hVEGF act by interfering with the binding of hVEGF to a cellular receptor, by incapacitating or killing cells which have been activated by hVEGF, or by interfering with vascular endothelial cell activation after hVEGF binding to a cellular receptor. All such points of intervention by an hVEGF antagonist shall be considered equivalent for purposes of this invention. Thus, included within the scope of the invention are antibodies, and preferably monoclonal antibodies, or fragments thereof, that bind to hVEGF, hVEGF receptor, or a complex comprising hVEGF in association with hVEGF receptor. Also included within the scope of the invention are fragments and amino acid sequence variants of hVEGF that bind to hVEGF receptor but which do not exhibit a biological activity of native hVEGF. Also included within the scope of the invention are hVEGF receptor and fragments and amino acid sequence variants thereof which are capable of binding hVEGF.

The term "hVEGF receptor" or "hVEGFr" as used herein refers to a cellular for hVEGF, ordinarily a cell-surface receptor found on vascular endothelial cells, as well as variants thereof which retain the ability to bind hVEGF. Typically, the hVEGF receptors and variants thereof that are hVEGF antagonists will be in isolated form, rather than being integrated into a cell membrane or fixed to a cell surface as may be the case in nature. One example of a hVEGF receptor is the fms-like tyrosine kinase (flt), a transmembrane receptor in the tyrosine kinase family. DeVries, et al., Science 255:989 (1992); Shibuya, et al., Oncogene 5:519 (1990). The flt receptor comprises an extracellular domain, a transmembrane domain, and an intracellular domain with tyrosine kinase activity. The extracellular domain is involved in the binding of hVEGF, whereas the intracellular domain is involved in signal transduction.

Another example of an hVEGF receptor is the flk-1 receptor (also referred to as KDR). Matthews, et al., Proc. Nat. Acad. Sci. 88:9026 (1991); Terman, et al., Oncogene 6:1677 (1991); Terman, et al., Biochem. Biophys. Res. Commun. 187:1579 (1992).

Binding of hVEGF to the flt receptor results in the formation of at least two high molecular weight complexes, having apparent molecular weight of 205,000 and 300,000 Daltons. The 300,000 Dalton complex is believed to be a dimer comprising two receptor molecules bound to a single molecule of hVEGF.

Variants of hVEGFr also are included within the scope hereof. Representative examples include truncated forms of a receptor in which the transmembrane and cytoplasmic domains are deleted from the receptor, and fusions proteins in which non-hVEGFr polymers or polypeptides are conjugated to the hVEGFr or, preferably, truncated forms thereof. An example of such a non-hVEGF polypeptide is an immunoglobulin. In that case, for example, the extracellular domain of the hVEGFr is substituted for the Fv domain of an immunoglobulin light or (preferably) heavy chain, with the C-terminus of the receptor extracellular domain covalently joined to the amino terminus of the CH1, hinge, CH2 or other fragment of the heavy chain. Such variants are made in the same fashion as known immunoadhesons. See e.g., Gascoigne, et al., Proc. Nat. Acad. Sci. 84:2936 (1987); Capon, et al., Nature 337:525 (1989); Aruffo, et al., Cell 61:1303 (1990); Ashkenazi, et al., Proc. Nat. Acad. Sci. 88:10535 (1991); Bennett, et al., J. Biol. Chem. 266:23060 (1991). In other embodiments, the hVEGFr is conjugated to a non-proteinaceous polymer such as polyethylene glycol (PEG) (see e.g., Davis, et al., U.S. Patent No. 4,179,337; Goodson, et al., BioTechnology 8:343-346 (1990); Abuchowski, et al., J. Biol. Chem. 252:3578 (1977); Abuchowski, et al., J. Biol. Chem. 252:3582 (1977)) or carbohydrates (see e.g., Marshall, et al., Arch. Biochem. Biophys., 167:77 (1975)). This serves to extend the biological half-life of the hVEGFr and reduces the possibility that the receptor will be immunogenic in the mammal to which it is administered. The hVEGFr is used in substantially the same fashion as antibodies to hVEGF, taking into account the affinity of the antagonist and its valency for hVEGF.

The extracellular domain of hVEGF receptor, either by itself or fused to an immunoglobulin polypeptide or other carrier polypeptide, is especially useful as an antagonist of hVEGF, by virtue of its ability to sequester hVEGF that is present in a host but that is not bound to hVEGFr on a cell surface.

hVEGFr and variants thereof also are useful in screening assays to identify agonists and antagonists of hVEGF. For example, host cells transfected with DNA encoding hVEGFr (for example, flt or flk1) overexpress the receptor polypeptide on the cell surface, making such recombinant host cells ideally suited for analyzing the ability of a test compound (for example, a small molecule, linear or cyclic peptide, or polypeptide) to bind to hVEGFr. hVEGFr and hVEGFr fusion proteins, such as an hVEGFr-IgG fusion protein, may be used in a similar fashion. For example, the fusion protein is bound to an immobilized support and the ability

of a test compound to displace radiolabeled hVEGF from the hVEGFr domain of the fusion protein is determined.

The term "recombinant" used in reference to hVEGF, hVEGF receptor, monoclonal antibodies, or other proteins, refers to proteins that are produced by recombinant DNA expression in a host cell. The host cell may be prokaryotic (for example, a bacterial cell such as E. coli) or eukaryotic (for example, a yeast or a mammalian cell).

Antagonist Monoclonal Antibodies

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical in specificity and affinity except for possible naturally occurring mutations that may be present in minor amounts. It should be appreciated that as a result of such naturally occurring mutations and the like, a monoclonal antibody composition of the invention, which will predominantly contain antibodies capable of specifically binding hVEGF, hVEGFr, or a complex comprising hVEGF in association with hVEGFr ("hVEGF-hVEGFr complex"), may also contain minor amounts of other antibodies.

Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from such a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, monoclonal antibodies of the invention may be made using the hybridoma method first described by Kohler & Milstein, Nature 256:495 (1975), or may be made by recombinant DNA methods. Cabilly, et al., U.S. Pat. No. 4,816,567.

In the hybridoma method, a mouse or other appropriate host animal is immunized with antigen by subcutaneous, intraperitoneal, or intramuscular routes to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein(s) used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986).

The antigen may be hVEGF, hVEGFr, or hVEGF-hVEGFr complex. The antigen optionally is a fragment or portion of any one of hVEGF or hVEGFr having one or more amino acid residues that participate in the binding of hVEGF to one of its receptors. For example, immunization with the extracellular domain of an hVEGFr (that is, a truncated hVEGFr polypeptide lacking transmembrane and intracellular domains) will be especially useful in producing antibodies that are antagonists of hVEGF, since it is the extracellular domain that is involved in hVEGF binding.

Monoclonal antibodies capable of binding hVEGF-hVEGFr complex are useful, particularly if they do not also bind to non-associated (non-complexed) hVEGF and hVEGFr. Such antibodies thus only bind to cells undergoing immediate activation by hVEGF and

accordingly are not sequestered by free hVEGF or hVEGFr as is normally found in a mammal. Such antibodies typically bind an epitope that spans one or more points of contact between the receptor and hVEGF. Such antibodies have been produced for other ligand receptor complexes and may be produced here in the same fashion. These antibodies need not, and may not, neutralize or inhibit a biological activity of non-associated hVEGF or hVEGFr, whether or not the antibodies are capable of binding to non-associated hVEGF or hVEGFr.

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, SP-2 cells available from the American Type Culture Collection, Rockville, Maryland USA, and P3X63Ag8U.1 cells described by Yelton, et al., Curr. Top. Microbiol. Immunol. 81:1 (1978). Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. Kozbor, J. Immunol. 133:3001 (1984). Brodeur, et al., Monoclonal Antibody Production Techniques and Applications, pp.51-63 (Marcel Dekker, Inc., New York, 1987).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). The monoclonal antibodies of the invention are those that preferentially immunoprecipitate hVEGF, hVEGFr, or hVEGF-hVEGFr complex, or that preferentially bind to at least one of those antigens in a binding assay, and that are capable of inhibiting a biological activity of hVEGF.

After hybridoma cells are identified that produce antagonist antibodies of the desired specificity, affinity, and activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods. Goding, Monoclonal Antibodies: Principles and Practice, pp.59-104 (Academic Press, 1986). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese Hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells.

The DNA optionally may be modified in order to change the character of the immunoglobulin produced by its expression. For example, humanized forms of murine antibodies are produced by substituting a complementarity determining region (CDR) of the murine antibody variable domain for the corresponding region of a human antibody. In some embodiments, selected framework region (FR) amino acid residues of the murine antibody also are substituted for the corresponding amino acid residues in the human antibody. Carter, et al., Proc. Nat. Acad. Sci. 89:4285 (1992); Carter, et al., BioTechnology 10:163 (1992). Chimeric forms of murine antibodies also are produced by substituting the coding sequence for selected human heavy and light constant chain domains in place of the homologous murine sequences. Cabilly, et al., U.S. Pat. No. 4,816,567; Morrison, et al., Proc. Nat. Acad. Sci. 81:6851 (1984).

The antibodies included within the scope of the invention include variant antibodies, such as chimeric (including "humanized") antibodies and hybrid antibodies comprising immunoglobulin chains capable of binding hVEGF, hVEGFr, or hVEGF-hVEGFr complex, and a non-hVEGF epitope.

The antibodies herein include all species of origin, and immunoglobulin classes (e.g., IgA, IgD, IgE, IgG, and IgM) and subclasses, as well as antibody fragments (e.g., Fab, $F(ab')_2$, and Fv), so long as they are capable of binding hVEGF, hVEGFr, or hVEGF-hVEGFr complex, and are capable of antagonizing a biological activity of hVEGF.

In a preferred embodiment of the invention, the monoclonal antibody will have an affinity for the immunizing antigen of at least about 10^9 liters/mole, as determined, for example, by the Scatchard analysis of Munson & Pollard, Anal. Biochem. 107:220 (1980). Also, the monoclonal antibody typically will inhibit the mitogenic or angiogenic activity of hVEGF at least about 50%, preferably greater than 80%, and most preferably greater than 90%, as determined, for example, by an in vitro cell survival or proliferation assay, such as described in Example 2.

For some therapeutic and diagnostic applications, it is desirable that the monoclonal antibody be reactive with fewer than all of the different molecular forms of hVEGF. For example, it may be desirable to have a monoclonal antibody that is capable of specifically binding to the 165-amino acid sequence hVEGF but not to the 121- or 189-amino acid sequence hVEGF polypeptides. Such antibodies are readily identified by comparative ELISA assays or comparative immunoprecipitation of the different hVEGF polypeptides.

Conjugates with Cytotoxic Moieties

In some embodiments it is desireable to provide a cytotoxic moiety conjugated to a hVEGF-specific monoclonal antibody or to hVEGFr. In these embodiments the cytotoxin serves to incapacitate or kill cells which are expressing or binding hVEGF or its receptor. The conjugate is targeted to the cell by the domain which is capable of binding to hVEGF, hVEGFr, or hVEGF-hVEGFr complex. Thus, monoclonal antibodies that are capable of binding hVEGF, hVEGFr, or hVEGF-hVEGFr complex are conjugated to cytotoxins. Similarly, hVEGFr is conjugated to a cytotoxin. While the monoclonal antibodies optimally are capable of neutralizing the activity of hVEGF alone (without the cytotoxin), it is not necessary in this embodiment that the monoclonal antibody or receptor be capable of any more than binding to hVEGF, hVEGFr, or hVEGF-hVEGFr complex.

Typically, the cytotoxin is a protein cytotoxin, e.g. diptheria, ricin or Pseudomonas toxin, although in the case of certain classes of immunoglobulins the Fc domain of the monoclonal antibody itself may serve to provide the cytotoxin (e.g., in the case of IgG2 antibodies, which are capable of fixing complement and participating in antibody-dependent cellular cytotoxicity (ADCC)). However, the cytotoxin does not need to be proteinaceous and may include chemotherapeutic agents heretofore employed, for example, for the treatment of tumors.

The cytotoxin typically is linked to a monoclonal antibody or fragment thereof by a backbone amide bond within (or in place of part or all of) the Fc domain of the antibody. Where the targeting function is supplied by hVEGFr, the cytotoxic moiety is substituted onto any domain of the receptor that does not participate in hVEGF binding; preferably, the moiety is substituted in place of or onto the transmembrane and or cytoplasmic domains of the receptor. The optimal site of substitution will be determined by routine experimentation and is well within the ordinary skill.

Conjugates which are protein fusions are easily made in recombinant cell culture by expressing a gene encoding the conjugate. Alternatively, the conjugates are made by covalently crosslinking the cytotoxic moiety to an amino acid residue side chain or C-terminal carboxyl of the antibody or the receptor, using methods known *per se* such as disulfide exchange or linkage through a thioester bond using for example iminothiolate and methyl-4-mercaptobutyrimadate.

Conjugates with other Moieties

The monoclonal antibodies and hVEGFr that are antagonists of hVEGF also are conjugated to substances that may not be readily classified as cytotoxins in their own right, but which augment the activity of the compositions herein. For example, monoclonal antibodies or hVEGFr capable of binding to hVEGF, hVEGFr, or hVEGF-hVEGFr complex are fused with heterologous polypeptides, such as viral sequences, with cellular receptors, with cytokines such as TNF, interferons, or interleukins, with polypeptides having procoagulant activity, and with other biologically or immunologically active polypeptides. Such fusions are readily made by recombinant methods. Typically such non-immunoglobulin polypeptides are substituted for the constant domain(s) of an anti-hVEGF or anti-hVEGF-hVEGFr complex antibody, or for the transmembrane and/or intracellular domain of an hVEGFr. Alternatively, they are substituted for a variable domain of one antigen binding site of an anti-hVEGF antibody described herein.

In preferred embodiments, such non-immunoglobulin polypeptides are joined to or substituted for the constant domains of an antibody described herein. Bennett, et al., J. Biol. Chem. 266:23060-23067 (1991). Alternatively, they are substituted for the Fv of an antibody herein to create a chimeric polyvalent antibody comprising at least one remaining antigen binding site having specificity for hVEGF, hVEGFr, or a hVEGF-hVEGFr complex, and a surrogate antigen binding site having a function or specificity distinct from that of the starting antibody.

Heterospecific Antibodies

Monoclonal antibodies capable of binding to hVEGF, hVEGFr, or hVEGF-hVEGFr complex need only contain a single binding site for the enumerated epitopes, typically a single heavy-light chain complex or fragment thereof. However, such antibodies optionally also bear antigen binding domains that are capable of binding an epitope not found within any one of hVEGF, hVEGFr, or hvEGF-hVEGFr complex. For example, substituting the corresponding amino acid sequence or amino acid residues of a native anti-hVEGF, anti-HVEGFr, or anti-hVEGF-hVEGFr complex antibody with the complementarity-determing and, if necessary, framework residues of an antibody having specificity for an antigen other than hVEGF, hVEGFr, or hVEGF-hVEGFr complex will create a polyspecific antibody comprising one antigen binding site having specificity for hVEGF, hVEGFr, or hVEGF-hVEGFr complex, and another antigen binding site having specificity for the non-hVEGF, hVEGFr, or hVEGF-hVEGFr complex antigen. These antibodies are at least bivalent, but may be polyvalent, depending upon the number of antigen binding sites possessed by the antibody class chosen. For example, antibodies of the IgM class will be polyvalent.

In preferred embodiments of the invention such antibodies are capable of binding an hVEGF or hVEGFr epitope and either (a) a polypeptide active in blood coagulation, such as protein C or tissue factor, (b) a cytotoxic protein such as tumor necrosis factor (TNF), or (c)

a non-hVEGFr cell surface receptor, such as CD4, or HER-2 receptor (Maddon, et al., Cell 42:93 (1985); Coussens, et al., Science 230:1137 (1985)). Heterospecific, multivalent antibodies are conveniently made by cotransforming a host cell with DNA encoding the heavy and light chains of both antibodies and thereafter recovering, by immunoaffinity chromatography or the like, the proportion of expressed antibodies having the desired antigen binding properties. Alternatively, such antibodies are made by in vitro recombination of monospecific antibodies.

Monovalent Antibodies

Monovalent antibodies capable of binding to hVEGFr or hVEGF-hVEGFr complex are especially useful as antagonists of hVEGF. Without limiting the invention to any particular mechanism of biological activity, it is believed that activation of cellular hVEGF receptors proceeds by a mechanism wherein the binding of hVEGF to cellular hVEGF receptors induces aggregation of the receptors, and in turn activates intracellular receptor kinase activity. Because monovalent anti-hVEGF receptor antibodies cannot induce such aggregation, and therefore cannot activate hVEGF receptor by that mechanism, they are ideal antagonists of hVEGF.

It should be noted, however, that these antibodies should be directed against the hVEGF binding site of the receptor or should otherwise be capable of interfering with hVEGF binding to the receptor hVEGF, such as by sterically hindering hVEGF access to the receptor. As described elsewhere herein, however, anti-hVEGFr antibodies that are not capable of interfering with hVEGF binding are useful when conjugated to non-immunoglobulin moieties, for example, cytotoxins.

Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking. In vitro methods are also suitable for preparing monovalent antibodies. For example, Fab fragments are prepared by enzymatic cleavage of intact antibody.

Diagnostic Uses

For diagnostic applications, the antibodies or hVEGFr of the invention typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; radioactive isotopic labels, such as, e.g., ^{125}I, ^{32}P, ^{14}C, or ^{3}H, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase.

Any method known in the art for separately conjugating the antibody or hVEGFr to the detectable moiety may be employed, including those methods described by Hunter, et al., Nature 144:945 (1962); David, et al., Biochemistry 13:1014 (1974); Pain, et al., J. Immunol. Meth. 40:219 (1981); and Nygren, J. Histochem. and Cytochem. 30:407 (1982).

The antibodies and receptors of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: A Manual of Techniques, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard (which may be hVEGF or an immunologically reactive portion thereof) to compete with the test sample analyte (hVEGF) for binding with a limited amount of antibody. The amount of hVEGF in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies or receptors. To facilitate determining the amount of standard that becomes bound, the antibodies or receptors generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies or receptors may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies or receptors, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody or receptor which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex. David & Greene, U.S. Pat No. 4,376,110. The second antibody or receptor may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

The antibodies or receptor herein also is useful for in vivo imaging, wherein an antibody or hVEGFr labeled with a detectable moiety is administered to a patient, preferably into the bloodstream, and the presence and location of the labeled antibody or receptor in the patient is assayed. This imaging technique is useful, for example, in the staging and treatment of neoplasms. The antibody or hVEGFr is labeled with any moiety that is detectable in a mammal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

Antagonist Variants of hVEGF

In addition to the antibodies described herein, other useful antagonists of hVEGF include fragments and amino acid sequence variants of native hVEGF that bind to hVEGF receptor but that do not exhibit the biological activity of native hVEGF. For example, such antagonists include fragments and amino acid sequence variants that comprise a receptor binding domain of hVEGF, but that lack a domain conferring biological activity, or that

otherwise are defective in activating cellular hVEGF receptors, such as in the case of a fragment or an amino acid sequence variant that is deficient in its ability to induce aggregation or activation of cellular hVEGF receptors. The term "receptor binding domain" refers to the amino acid sequences in hVEGF that are involved in hVEGF receptor binding. The term "biological activity domain" or "domain conferring biological activity" refers to an amino acid sequence in hVEGF that confer a particular biological activity of the factor, such as mitogenic or angiogenic activity.

The observation that hVEGF appears to be capable of forming a complex with two or more hVEGFr molecules on the surface of a cell suggests that hVEGF has at least two discrete sites for binding to hVEGFr and that it binds to such cellular receptors in sequential fashion, first at one site and then at the other before activation occurs, in the fashion of growth hormone, prolactin and the like (see e.g., Cunningham, et al., Science 254:821 (1991); deVos, et al., Science 255:306 (1992); Fuh, et al., Science 256:1677 (1992)). Accordingly, antagonist variants of hVEGF are selected in which one receptor binding site of hVEGF (typically the site involved in the initial binding of hVEGF to hVEGFr) remains unmodified (or if modified is varied to enhance binding), while a second receptor binding site of hVEGF typically is modified by nonconservative amino acid residue substitution(s) or deletion(s) in order to render that binding site inoperative.

Receptor binding domains in hVEGF and hVEGF binding domains in hVEGFr are determined by methods known in the art, including X-ray studies, mutational analyses, and antibody binding studies. The mutational approaches include the techniques of random saturation mutagenesis coupled with selection of escape mutants, and insertional mutagenesis. Another strategy suitable for identifying receptor-binding domains in ligands is known as alanine (Ala)-scanning mutagenesis. Cunningham, et al., Science 244, 1081-1985 (1989). This method involves the identification of regions that contain charged amino acid side chains. The charged residues in each region identified (i.e. Arg, Asp, His, Lys, and Glu) are replaced (one region per mutant molecule) with Ala and the receptor binding of the obtained ligands is tested, to assess the importance of the particular region in receptor binding. A further powerful method for the localization of receptor binding domains is through the use of neutralizing anti-hVEGF antibodies. Kim, et al., Growth Factors 7:53 (1992). Usually a combination of these and similar methods is used for localizing the domains involved in receptor binding.

The term "amino acid sequence variant" used in reference to hVEGF refers to polypeptides having amino acid sequences that differ to some extent from the amino acid sequences of the native forms of hVEGF. Ordinarily, antagonist amino acid sequence variants will possess at least about 70% homology with at least one receptor binding domain of a native hVEGF, and preferably, they will be at least about 80%, more preferably at least about 90% homologous with a receptor binding domain of a native hVEGF. The amino acid

sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of native hVEGF, such that the variants retain the ability to bind to hVEGF receptor (and thus compete with native hVEGF for binding to hVEGF receptor) but fail to induce one or more of the biological effects of hVEGF, such as endothelial cell proliferation, angiogenesis, or vascular permeability.

"Homology" is defined as the percentage of residues in the amino acid sequence variant that are identical with the residues in the amino acid sequence of a receptor binding domain of a native hVEGF after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2", authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, DC 20559, on December 10, 1991. Substitutional variants are those that have at least one amino acid residue in a native sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

Insertional variants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a native sequence. Immediately adjacent to an amino acid means connected to either the α-carboxy or α-amino functional group of the amino acid.

Deletional variants are those with one or more amino acid residues in a native sequence removed. Ordinarily, deletional variants will have one or two amino acid residues deleted in a particular region of the molecule.

Fragments and amino acid sequence variants of hVEGF are readily prepared by methods known in the art, such as by site directed mutagenesis of the DNA encoding the native factor. The mutated DNA is inserted into an appropriate expression vector, and host cells are then transfected with the recombinant vector. The recombinant host cells and grown in suitable culture medium, and the desired fragment or amino acid sequence variant expressed in the host cells then is recovered from the recombinant cell culture by chromatographic or other purification methods.

Alternatively, fragments and amino acid variants of hVEGF are prepared in vitro, for example by proteolysis of native hVEGF, or by synthesis using standard solid-phase peptide synthesis procedures as described by Merrifield (J. Am. Chem. Soc. 85:2149 [1963]), although other equivalent chemical syntheses known in the art may be used. Solid-phase synthesis is initiated from the C-terminus of the peptide by coupling a protected α-amino acid to a suitable resin. The amino acids are coupled to the peptide chain using techniques well known in the art for the formation of peptide bonds.

Therapeutic Uses

For therapeutic applications, the antagonists of the invention are administered to a mammal, preferably a human, in a pharmaceutically acceptable dosage form, including those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intra-cerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The antagonists also are suitably administered by intratumoral, peritumoral, intralesional, or perilesional routes, to exert local as well as systemic therapeutic effects. The intraperitoneal route is expected to be particularly useful, for example, in the treatment of ovarian tumors.

Such dosage forms encompass pharmaceutically acceptable carriers that are inherently nontoxic and nontherapeutic. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and polyethylene glycol. Carriers for topical or gel-based forms of antagonist include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wood wax alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations. The antagonist will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml.

Suitable examples of sustained release preparations include semipermeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate) as described by Langer et al., J. Biomed. Mater. Res. 15:167 (1981) and Langer, Chem. Tech., 12: 98-105 (1982), or poly(vinylalcohol), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., Biopolymers, 22:547 (1983), non-degradable ethylene-vinyl acetate (Langer et al., supra), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microperes composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated polypeptide antagonists remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and

possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release hVEGF antagonist compositions also include liposomally entrapped antagonist antibodies and hVEGFr. Liposomes containing the antagonists are prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA, 82:3688 (1985); Hwang, et al., Proc. Natl. Acad. Sci. USA, 77:4030 (1980); U.S. Patent No. 4,485,045; U.S. Patent No. 4,544,545. Ordinarily the liposomes are the small (about 200-800 Angstroms) unilamelar type in which the lipid content is greater than about 30 mol.% cholesterol, the selected proportion being adjusted for the optimal HRG therapy. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Another use of the present invention comprises incorporating an hVEGF antagonist into formed articles. Such articles can be used in modulating endothelial cell growth and angiogenesis. In addition, tumor invasion and metastasis may be modulated with these articles.

For the prevention or treatment of disease, the appropriate dosage of antagonist will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibodies are administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antagonist, and the discretion of the attending physician. The antagonist is suitably administered to the patient at one time or over a series of treatments.

The hVEGF antagonists are useful in the treatment of various neoplastic and non-neoplastic diseases and disorders. Neoplasms and related conditions that are amenable to treatment include breast carcinomas, lung carcinomas, gastric carcinomas, esophageal carcinomas, colorectal carcinomas, liver carcinomas, ovarian carcinomas, thecomas, arrhenoblastomas, cervical carcinomas, endometrial carcinoma, endometrial hyperplasia, endometriosis, fibrosarcomas, choriocarcinoma, head and neck cancer, nasopharyngeal carcinoma, laryngeal carcinomas, hepatoblastoma, Kaposi's sarcoma, melanoma, skin carcinomas, hemangioma, cavernous hemangioma, hemangioblastoma, pancreas carcinomas, retinoblastoma, astrocytoma, glioblastoma, Schwannoma, oligodendroglioma, medulloblastoma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, thyroid carcinomas, Wilm's tumor, renal cell carcinoma, prostate carcinoma, abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

Non-neoplastic conditions that are amenable to treatment include rheumatoid arthritis, psoriasis, atherosclerosis, diabetic and other retinopathies, retrolental fibroplasia, neovascular glaucoma, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, chronic inflammation, lung inflammation, nephrotic syndrome, preeclampsia, ascites, pericardial effusion (such as that associated with pericarditis), and pleural effusion.

Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg of antagonist is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays, including, for example, radiographic tumor imaging.

According to another embodiment of the invention, the effectiveness of the antagonist in preventing or treating disease may be improved by administering the antagonist serially or in combination with another agent that is effective for those purposes, such as tumor necrosis factor (TNF), an antibody capable of inhibiting or neutralizing the angiogenic activity of acidic or basic fibroblast growth factor (FGF) or hepatocyte growth factor (HGF), an antibody capable of inhibiting or neutralizing the coagulant activities of tissue factor, protein C, or protein S (see Esmon, et al., PCT Patent Publication No. WO 91/01753, published 21 February 1991), or one or more conventional therapeutic agents such as, for example, alkylating agents, folic acid antagonists, anti-metabolites of nucleic acid metabolism, antibiotics, pyrimidine analogs, 5-fluorouracil, purine nucleosides, amines, amino acids, triazol nucleosides, or corticosteroids. Such other agents may be present in the composition being administered or may be administered separately. Also, the antagonist is suitably administered serially or in combination with radiological treatments, whether involving irradiation or administration of radioactive substances.

In one embodiment, vascularization of tumors is attacked in combination therapy. One or more hVEGF antagonists are administered to tumor-bearing patients at therapeutically effective doses as determined for example by observing necrosis of the tumor or its metastatic foci, if any. This therapy is continued until such time as no further beneficial effect is observed or clinical examination shows no trace of the tumor or any metastatic foci. Then TNF is administered, alone or in combination with an auxiliary agent such as alpha-, beta-, or gamma-interferon, anti-HER2 antibody, heregulin, anti-heregulin antibody, D-factor, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte-macrophage colony stimulating factor (GM-CSF), or agents that promote microvascular coagulation in tumors, such as anti-protein

C antibody, anti-protein S antibody, or C4b binding protein (see Esmon, et al., PCT Patent Publication No. WO 91/01753, published 21 February 1991), or heat or radiation.

Since the auxiliary agents will vary in their effectiveness it is desireable to compare their impact on the tumor by matrix screening in conventional fashion. The administration of hVEGF antagonist and TNF is repeated until the desired clinical effect is achieved. Alternatively, the hVEGF antagonist(s) are administered together with TNF and, optionally, auxiliary agent(s). In instances where solid tumors are found in the limbs or in other locations susceptible to isolation from the general circulation, the therapeutic agents described herein are administered to the isolated tumor or organ. In other embodiments, a FGF or platelet-derived growth factor (PDGF) antagonist, such as an anti-FGF or an anti-PDGF neutralizing antibody, is administered to the patient in conjunction with the hVEGF antagonist. Treatment with hVEGF antagonists optimally may be suspended during periods of wound healing or desirable neovascularization.

Other Uses

The anti-hVEGF antibodies of the invention also are useful as affinity purification agents. In this process, the antibodies against hVEGF are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the hVEGF to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the hVEGF, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the hVEGF from the antibody.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner.

EXAMPLE 1

Preparation of Anti- hVEGF Monoclonal Antibodies

To obtain hVEGF conjugated to keyhole limpet hemocyanin (KLH) for immunization, recombinant hVEGF (165 amino acids), Leung, et al., Science 246:1306 (1989), was mixed with KLH at a 4:1 ratio in the presence of 0.05% glutaraldehyde and the mixture was incubated at room temperature for 3 hours with gentle stirring. The mixture then was dialyzed against phosphate buffered saline (PBS) at 4° C. overnight.

Balb/c mice were immunized four times every two weeks by intraperitoneal injections with 5 μg of hVEGF conjugated to 20 μg of KLH, and were boosted with the same dose of hVEGF conjugated to KLH four days prior to cell fusion.

Spleen cells from the immunized mice were fused with P3X63Ag8U.1 myeloma cells, Yelton, et al., Curr. Top. Microbiol. Immunol. 81:1 (1978), using 35% polyethylene glycol (PEG) as described. Yarmush, et al., Proc. Nat. Acad. Sci. 77:2899 (1980). Hybridomas were selected in HAT medium.

Supernatants from hybridoma cell cultures were screened for anti-hVEGF antibody production by an ELISA assay using hVEGF-coated microtiter plates. Antibody that was bound to hVEGF in each of the wells was determined using alkaline phosphatase-conjugated goat anti-mouse IgG immunoglobulin and the chromogenic substrate p-nitrophenyl phosphate. Harlow & Lane, Antibodies: A Laboratory Manual, p.597 (Cold Spring Harbor Laboratory, 1988). Hybridoma cells thus determined to produce anti-hVEGF antibodies were subcloned by limiting dilution, and two of those clones, designated A4.6.1 and B2.6.2, were chosen for further studies.

EXAMPLE 2

Characterization of Anti-hVEGF Monoclonal Antibodies

A. Antigen Specificity

The binding specificities of the anti-hVEGF monoclonal antibodies produced by the A4.6.1 and B2.6.2 hybridomas were determined by ELISA. The monoclonal antibodies were added to the wells of microtiter plates that previously had been coated with hVEGF, FGF, HGF, or epidermal growth factor (EGF). Bound antibody was detected with peroxidase conjugated goat anti-mouse IgG immunoglobulins. The results of those assays confirmed that the monoclonal antibodies produced by the A4.6.1 and B2.6.2 hybridomas bind to hVEGF, but not detectably to those other protein growth factors.

B. Epitope Mapping

A competitive binding ELISA was used to determine whether the monoclonal antibodies produced by the A4.6.1 and B2.6.2 hybridomas bind to the same or different epitopes (sites) within hVEGF. Kim, et al., Infect. Immun. 57:944 (1989). Individual unlabeled anti-hVEGF monoclonal antibodies (A4.6.1 or B2.6.2) or irrelevant anti-HGF antibody (IgG1 isotype) were added to the wells of microtiter plates that previously had been coated with hVEGF. Biotinylated anti-hVEGF monoclonal antibodies (BIO-A4.6.1 or BIO-B2.6.2) were then added. The ratio of biotinylated antibody to unlabeled antibody was 1:1000. Binding of the biotinylated antibodies was visualized by the addition of avidin-conjugated peroxidase, followed by o-phenylenediamine dihydrochloride and hydrogen peroxide. The color reaction, indicating the amount of biotinylated antibody bound, was determined by measuring the optical density (O.D) at 495 nm wavelength.

As shown in Figure 1, in each case, the binding of the biotinylated anti-hVEGF antibody was inhibited by the corresponding unlabeled antibody, but not by the other unlabeled anti-hVEGF antibody or the anti-HGF antibody. These results indicate that the monoclonal antibodies produced by the A4.6.1 and B2.6.2 hybridomas bind to different epitopes within hVEGF.

C. Isotyping

The isotypes of the anti-hVEGF monoclonal antibodies produced by the A4.6.1 and B2.6.2 hybridomas were determined by ELISA. Samples of culture medium (supernatant) in

which each of the hybridomas was growing were added to the wells of microtiter plates that had previously been coated with hVEGF. The captured anti-hVEGF monoclonal antibodies were incubated with different isotype-specific alkaline phosphatase-conjugated goat anti-mouse immunoglobulins, and the binding of the conjugated antibodies to the anti-hVEGF monoclonal antibodies was determined by the addition of p-nitrophenyl phosphate. The color reaction was measured at 405 nm with an ELISA plate reader.

By that method, the isotype of the monoclonal antibodies produced by both the A4.6.1 and B2.6.2 hybridomas was determined to be IgG1.

D. Binding Affinity

The affinities of the anti-hVEGF monoclonal antibodies produced by the A4.6.1 and B2.6.2 hybridomas for hVEGF were determined by a competitive binding assays. A predetermined sub-optimal concentration of monoclonal antibody was added to samples containing 20,000 - 40,000 cpm ^{125}I-hVEGF (1 - 2 ng) and various known amounts of unlabeled hVEGF (1 - 1000 ng). After 1 hour at room temperature, 100 μl of goat anti-mouse Ig antisera (Pel-Freez, Rogers, AR USA) were added, and the mixtures were incubated another hour at room temperature. Complexes of antibody and bound protein (immune complexes) were precipitated by the addition of 500 μl of 6% polyethylene glycol (PEG, mol. wt. 8000) at 4° C., followed by centrifugation at 2000 x G. for 20 min. at 4° C. The amount of ^{125}I-hVEGF bound to the anti-hVEGF monoclonal antibody in each sample was determined by counting the pelleted material in a gamma counter.

Affinity constants were calculated from the data by Scatchard analysis. The affinity of the anti-hVEGF monoclonal antibody produced by the A4.6.1 hybridoma was calculated to be 1.2 x 10^9 liters/mole. The affinity of the anti-hVEGF monoclonal antibody produced by the B2.6.2 hybridoma was calculated to be 2.5 x 10^9 liters/mole.

E. Inhibition of hVEGF Mitogenic Activity

Bovine adrenal cortex capillary endothelial (ACE) cells, Ferrara, et al., Proc. Nat. Acad. Sci. 84:5773 (1987), were seeded at a density of 10^4 cells/ml in 12 multiwell plates, and 2.5 ng/ml hVEGF was added to each well in the presence or absence of various concentrations of the anti-hVEGF monoclonal antibodies produced by the A4.6.1 or B2.6.2 hybridomas, or an irrelevant anti-HGF monoclonal antibody. After culturing 5 days, the cells in each well were counted in a Coulter counter. As a control, ACE cells were cultured in the absence of added hVEGF.

As shown in Figure 2, both of the anti-hVEGF monoclonal antibodies inhibited the ability of the added hVEGF to support the growth or survival of the bovine ACE cells. The monoclonal antibody produced by the A4.6.1 hybridoma completely inhibited the mitogenic activity of hVEGF (greater than about 90% inhibition), whereas the monoclonal antibody produced by the B2.6.2 hybridoma only partially inhibited the mitogenic activity of hVEGF.

F. Inhibition of hVEGF Binding

Bovine ACE cells were seeded at a density of 2.5 x 10^4 cells/0.5 ml/well in 24 well microtiter plates in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% calf serum, 2 mM glutamine, and 1 ng/ml basic fibroblast growth factor. After culturing overnight, the cells were washed once in binding buffer (equal volumes of DMEM and F12 medium plus 25 mM HEPES and 1% bovine serum albumin) at 4° C.

12,000 cpm ^{125}I-hVEGF (approx. 5 x 10^4 cpm/ng/ml) was preincubated for 30 minutes with 5 μg of the anti-hVEGF monoclonal antibody produced by the A4.6.1, B2.6.2, or A2.6.1 hybridoma (250 μl total volume), and thereafter the mixtures were added to the bovine ACE cells in the microtiter plates. After incubating the cells for 3 hours at 4° C., the cells were washed 3 times with binding buffer at 4° C., solubilized by the addition of 0.5 ml 0.2 N. NaOH, and counted in a gamma counter.

As shown in Figure 3 (upper), the anti-hVEGF monoclonal antibodies produced by the A4.6.1 and B2.6.2 hybridomas inhibited the binding of hVEGF to the bovine ACE cells. In contrast, the anti-hVEGF monoclonal antibody produced by the A2.6.1 hybridoma had no apparent effect on the binding of hVEGF to the bovine ACE cells. Consistent with the results obtained in the cell proliferation assay described above, the monoclonal antibody produced by the A4.6.1 hybridoma inhibited the binding of hVEGF to a greater extent than the monoclonal antibody produced by the B2.6.2 hybridoma.

As shown in Figure 3 (lower), the monoclonal antibody produced by the A4.6.1 hybridoma completely inhibited the binding of hVEGF to the bovine ACE cells at a 1:250 molar ratio of hVEGF to antibody.

G. Cross-reactivity with other VEGF isoforms

To determine whether the anti-hVEGF monoclonal antibody produced by the A4.6.1 hybridoma is reactive with the 121- and 189-amino acid forms of hVEGF, the antibody was assayed for its ability to immunoprecipate those polypeptides.

Human 293 cells were transfected with vectors comprising the nucleotide coding sequence of the 121- and 189-amino acid hVEGF polypeptides, as described. Leung, et al., Science 246:1306 (1989). Two days after transfection, the cells were transferred to medium lacking cysteine and methionine. The cells were incubated 30 minutes in that medium, then 100 μCi/ml of each ^{35}S-methionine and ^{35}S-cysteine were added to the medium, and the cells were incubated another two hours. The labeling was chased by transferring the cells to serum free medium and incubating three hours. The cell culture media were collected, and the cells were lysed by incubating for 30 minutes in lysis buffer (150 mM NaCl, 1% NP40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 mM Tris, pH 8.0). Cell debris was removed from the lysates by centrifugation at 200 x G. for 30 minutes.

500 μl samples of cell culture media and cell lysates were incubated with 2 μl of A4.6.1 hybridoma antibody (2.4 mg/ml) for 1 hour at 4° C., and then were incubated with

5 µl of rabbit anti-mouse IgG immunoglobulin for 1 hour at 4° C. Immune complexes of ^{35}S-labeled hVEGF and anti-hVEGF monoclonal antibody were precipitated with protein-A Sepharose (Pharmacia), then subjected to SDS - 12% polyacrylamide gel electrophoresis under reducing conditions. The gel was exposed to x-ray film for analysis of the immunoprecipitated, radiolabeled proteins by autoradiography.

The results of that analysis indicated that the anti-hVEGF monoclonal antibody produced by the A4.6.1 hybridoma was cross-reactive with both the 121- and 189-amino acid forms of hVEGF.

EXAMPLE 3

Preparation of hVEGF Receptor - IgG Fusion Protein

The nucleotide and amino acid coding sequences of the flt hVEGF receptor are disclosed in Shibuya, et al., Oncogene 5:519-524 (1990). The coding sequence of the extracellular domain of the flt hVEGF receptor was fused to the coding sequence of human IgG1 heavy chain in a two-step process.

Site-directed mutagenesis was used to introduce a BstBI restriction into DNA encoding flt at a site 5′ to the codon for amino acid 759 of flt, and to convert the unique BssHII restriction site in plasmid pBSSK⁺FC, Bennett, et al., J. Biol. Chem. 266:23060-23067 (1991), to a BstBI site. The modified plasmid was digested with EcoRI and BstBI and the resulting large fragment of plasmid DNA was ligated together with an EcoRI-BstBI fragment of the flt DNA encoding the extracellular domain (amino acids 1-758) of the flt hVEGF receptor.

The resulting construct was digested with ClaI and NotI to generate an approximately 3.3 kb fragment, which is then inserted into the multiple cloning site of the mammalian expression vector pHEBO2 (Leung, et al., Neuron 8:1045 (1992) by ligation. The ends of 3.3. kb fragment are modified, for example by the addition of linkers, to obtain insertion of the fragment into the vector in the correct orientation for expression.

Mammalian host cells (for example, CEN4 cells (Leung, et al. supra) are transfected with the pHEBO2 plasmid containing the flt insert by electroporation. Transfected cells are cultured in medium containing about 10% fetal bovine serum, 2 mM glutamine, and antibiotics, and at about 75% confluency are transferred to serum free medium. Medium is conditioned for 3-4 days prior to collection, and the flt-IgG fusion protein is purified from the conditioned medium by chromatography on a protein-A affinity matrix essentially as described in Bennett, et al., J. Biol. Chem. 266:23060-23067 (1991).

EXAMPLE 4

Inhibition of Tumor Growth with hVEGF Antagonists

Various human tumor cell lines growing in culture were assayed for production of hVEGF by ELISA. Ovary, lung, colon, gastric, breast, and brain tumor cell lines were found

to produce hVEGF. Three cell lines that produced hVEGF, NEG 55 (also referred to as G55) (human glioma cell line obtained from Dr. M. Westphal, Department of Neurosurgery, University Hospital Eppendor, Hamburg, Germany, also referred to as G55), A-673 (human rhabdomyosarcoma cell line obtained from the American Type Culture Collection (ATCC), Rockville, Maryland USA 20852 as cell line number CRL 1598), and SK-LMS-1 (leiomyosarcoma cell line obtained from the ATCC as cell line number HTB 88), were used for further studies.

Six to ten week old female Beige/nude mice (Charles River Laboratory, Wilmington, Massachusetts USA) were injected subcutaneously with 1 - 5 x 10^6 tumor cells in 100-200 μl PBS. At various times after tumor growth was established, mice were injected intraperitoneally once or twice per week with various doses of A4.6.1 anti-hVEGF monoclonal antibody, an irrelevant anti-gp120 monoclonal antibody (5B6), or PBS. Tumor size was measured every week, and at the conclusion of the study the tumors were excised and weighed.

The effect of various amounts of A4.6.1 anti-hVEGF monoclonal antibody on the growth of NEG 55 tumors in mice is shown in Figures 4 and 5. Figure 4 shows that mice treated with 25 μg or 100 μg of A4.6.1 anti-hVEGF monoclonal antibody beginning one week after inoculation of NEG 55 cells had a substantially reduced rate of tumor growth as compared to mice treated with either irrelevant antibody or PBS. Figure 5 shows that five weeks after inoculation of the NEG 55 cells, the size of the tumors in mice treated with A4.6.1 anti-hVEGF antibody was about 50% (in the case of mice treated with 25 μg dosages of the antibody) to 85% (in the case of mice treated with 100 μg dosages of the antibody) less than the size of tumors in mice treated with irrelevant antibody or PBS.

The effect of A4.6.1 anti-hVEGF monoclonal antibody treatment on the growth of SK-LMS-1 tumors in mice is shown in Figure 6. Five weeks after innoculation of the SK-LMS-1 cells, the average size of tumors in mice treated with the A4.6.1 anti-hVEGF antibody was about 75% less than the size tumors in mice treated with irrelevant antibody or PBS.

The effect of A4.6.1 anti-hVEGF monoclonal antibody treatment on the growth of A673 tumors in mice is shown in Figure 7. Four weeks after innoculation of the A673 cells, the average size of tumors in mice treated with A4.6.1 anti-hVEGF antibody was about 60% (in the case of mice treated with 10 μg dosages of the antibody) to greater than 90% (in the case of mice treated with 50-400 μg dosages of the antibody) less than the size of tumors in mice treated with irrelevant antibody or PBS.

EXAMPLE 5

Analysis of the Direct Effect of Anti-hVEGF Antibody on Tumor Cells Growing in Culture

NEG55 human glioblastoma cells or A673 rhabdomyosarcoma cells were seeded at a density of 7 x 10^3 cells/well in multiwells plates (12 wells/plate) in F12/DMEM medium

containing 10% fetal calf serum, 2mM glutamine, and antibiotics. A4.6.1 anti-hVEGF antibody then was added to the cell cultures to a final concentration of 0 - 20.0 μg antibody/ml. After five days, the cells growing in the wells were dissociated by exposure to trypsin and counted in a Coulter counter.

Figures 8 and 9 show the results of those studies. As is apparent, the A4.6.1 anti-hVEGF antibody did not have any significant effect on the growth of the NEG55 or A673 cells in culture. These results indicate that the A4.6.1 anti-hVEGF antibody is not cytotoxic, and strongly suggest that the observed anti-tumor effects of the antibody are due to its inhibition of VEGF-mediated neovascularization.

EXAMPLE 6

Effect of Anti-hVEGF Antibody on
Endothelial Cell Chemotaxis

Chemotaxis of endothelial cells and others cells, including monocytes and lymphocytes, play an important role in the pathogenesis of rheumatoid arthritis. Endothelial cell migration and proliferation accompany the angiogenesis that occurs in the rheumatoid synovium. Vascularized tissue (pannus) invades and destroys the articular cartilage.

To determine whether hVEGF antagonists interfere with this process, we assayed the effect of the A4.6.1 anti-hVEGF antibody on endothelial cell chemotaxis stimulated by synovial fluid from patients having rheumatoid arthritis. As a control, we also assayed the effect of the A4.6.1 anti-hVEGF antibody on endothelial cell chemotaxis stimulated by synovial fluid from patients having osteoarthritis (the angiogenesis that occurs in rheumatoid arthritis does not occur in osteoarthritis).

Endothelial cell chemotaxis was assayed using modified Boyden chambers according to established procedures. Thompson, et al., Cancer Res. 51:2670 (1991); Phillips, et al., Proc. Exp. Biol. Med. 197:458 (1991). About 10^4 human umbilical vein endothelial cells were allowed to adhere to gelatin-coated filters (0.8 micron pore size) in 48-well multiwell microchambers in culture medium containing 0.1% fetal bovine serum. After about two hours, the chambers were inverted and test samples (rheumatoid arthritis synovial fluid, osteoarthritis synovial fluid, basic FGF (bFGF) (to a final concentration of 1 μg/ml), or PBS) and A4.6.1 anti-hVEGF antibody (to a final concentration of 10 μg/ml) were added to the wells. After two to four hours, cells that had migrated were stained and counted.

Figure 10 shows the averaged results of those studies. The values shown in the column labeled "Syn. Fluid" and shown at the bottom of the page for the controls are the average number of endothelial cells that migrated in the presence of synovial fluid, bFGF, or PBS alone. The values in the column labeled "Syn. Fluid + mAB VEGF" are the average number of endothelial cells that migrated in the presence of synovial fluid plus added A4.6.1 anti-hVEGF antibody. The values in the column labeled "% Suppression" indicate the percentage reduction in synovial fluid-induced endothelial cell migration resulting from the

addition of anti-hVEGF antibody. As indicated, the anti-hVEGF antibody significantly inhibited the ability of rheumatoid arthritis synovial fluid (53.40 average percentage inhibition), but not osteorthritis synovial fluid (13.64 average percentage inhibition), to induce endothelial cell migration.

What is claimed is:

1. A composition comprising a hVEGF antagonist, provided however that the antagonist is not the flt or flk-1 or KDR receptor or a neutralizing anti-hVEGF antibody.

2. A composition of claim 1 including a polypeptide comprising an antibody amino acid sequence that is capable of binding to a hVEGF receptor and that competes with hVEGF for binding to the receptor.

3. A composition of claim 1 including a polypeptide comprising an antibody amino acid sequence that is capable of binding to hVEGF and that interferes with the binding of hVEGF to a hVEGF receptor.

4. A monoclonal antibody amino acid sequence capable of specifically binding to a hVEGFr or a hVEGF-hVEGFr complex.

5. A monoclonal antibody amino acid sequence of claim 4 which inhibits the mitogenic activity of a hVEGF or inhibits the binding of a hVEGF to bovine ACE cells.

6. A monoclonal antibody amino acid sequence of claim 5 which inhibits the mitogenic activity of a hVEGF at least about 90%.

7. A monoclonal antibody amino acid sequence of claim 4 which is capable of binding to hVEGFr.

8. A monoclonal antibody amino acid sequence of claim 7 which is monovalent for binding to hVEGFr.

9. A monoclonal antibody amino acid sequence of claim 4 which is heterospecific.

10. A monoclonal antibody sequence of claim 9 which is capable of binding to an antigen other than hVEGF, hVEGFr, and hVEGF-hVEGFr complex.

11. A monoclonal antibody amino acid sequence of claim 4 which comprises an amino acid sequence from the Fc domain of either the IgA, IgD, IgE, IgG1, IgG2, IgG3, IgG4 or IgM heavy chains.

12. A monoclonal antibody amino acid sequence of claim 4 which comprises a human Fc domain.

13. A monoclonal antibody amino acid sequence of claim 12 which further comprises a murine Fv domain capable of binding hVEGF, hVEGFr, or hVEGF-hVEGFr complex.

14. A monoclonal antibody amino acid sequence of claim 4 further comprising a non-immunoglobulin polymer.

15. A monoclonal antibody amino acid sequence of claim 4 further comprising a cytotoxic moiety or an amino acid sequence of a cytokine.

16. A monoclonal antibody amino acid sequence of claim 15 wherein the cytotoxic moiety or the amino acid sequence of the cytokine is substituted for an Fc sequence.

17. A monoclonal antibody amino acid sequence of claim 15 having a cytotoxic moiety that is a polypeptide toxin.

18. A monoclonal antibody amino acid sequence of claim 15 having a cytotoxic moiety that is capable of Fc effector function or of recruiting an immune cell.

19. A monoclonal antibody amino acid sequence of claim 18 wherein the cytoxic moiety is a polypeptide capable of binding complement.

20. A monoclonal antibody amino acid sequence of claim 18 wherein the cytotoxic moiety is a polypeptide capable of binding CD3, CD18, CD11a, CDIIb, or CD11c.

21. A monoclonal antibody amino acid sequence of claim 4 which is capable of binding to hVEGF-hVEGFr complex but not to hVEGF or to hVEGFr alone.

22. A monoclonal antibody amino acid sequence of claim 21 further comprising a cytotoxic moiety.

23. A monoclonal antibody amino acid sequence of claim 4 which is capable of binding to hVEGFr and which antagonizes the effect of hVEGF on the hVEGFr.

24. A monoclonal antibody amino acid sequence of claim 4 further comprising a physiologically acceptable vehicle and which is sterile, present in a substantially isotonic solution, and stored in a container hermetically sealed with an elastomeric stopper.

25. A monoclonal antibody sequence of claim 24 in a kit together with a written insert containing instructions for therapeutic use.

26. A polypeptide comprising an amino acid sequence encoding a hVEGFr and an immunoglobulin chain.

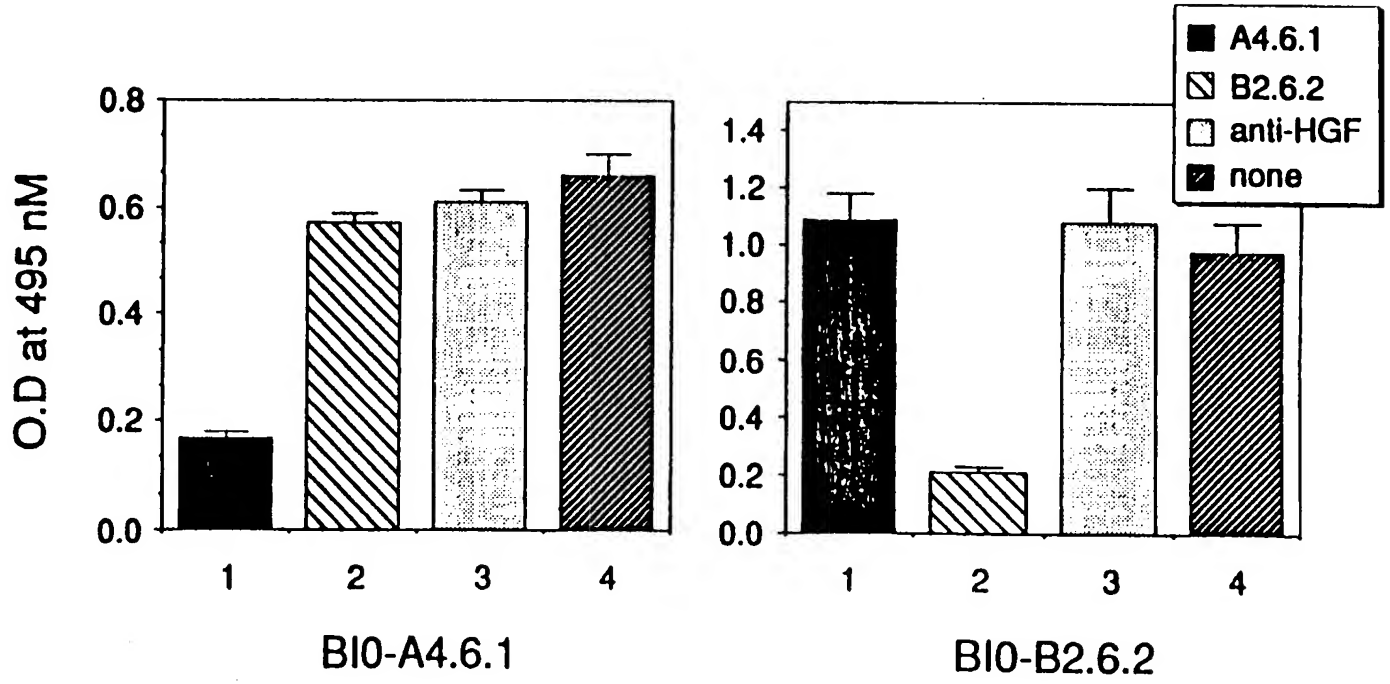
27. A method of treatment of a tumor in a mammal comprising administering to the mammal a therapeutically effective amount of a hVEGF antagonist sufficient to reduce the size of the tumor.

28. A method of claim 27 wherein the hVEGF antagonist is an anti-hVEGFr antibody.

29. A method of claim 27 wherein the hVEGF antagonist is an anti-hVEGF-hVEGFr complex antibody.

30. A method of claim 27 wherein the hVEGF antagonist comprises an amino acid sequence encoding the extracellular domain of a hVEGFr.

FIGURE 1


A4.6.1
B2.6.2
anti-HGF
none
O.D at 495 nM
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1
2
3
4
BI0-A4.6.1
BI0-B2.6.2

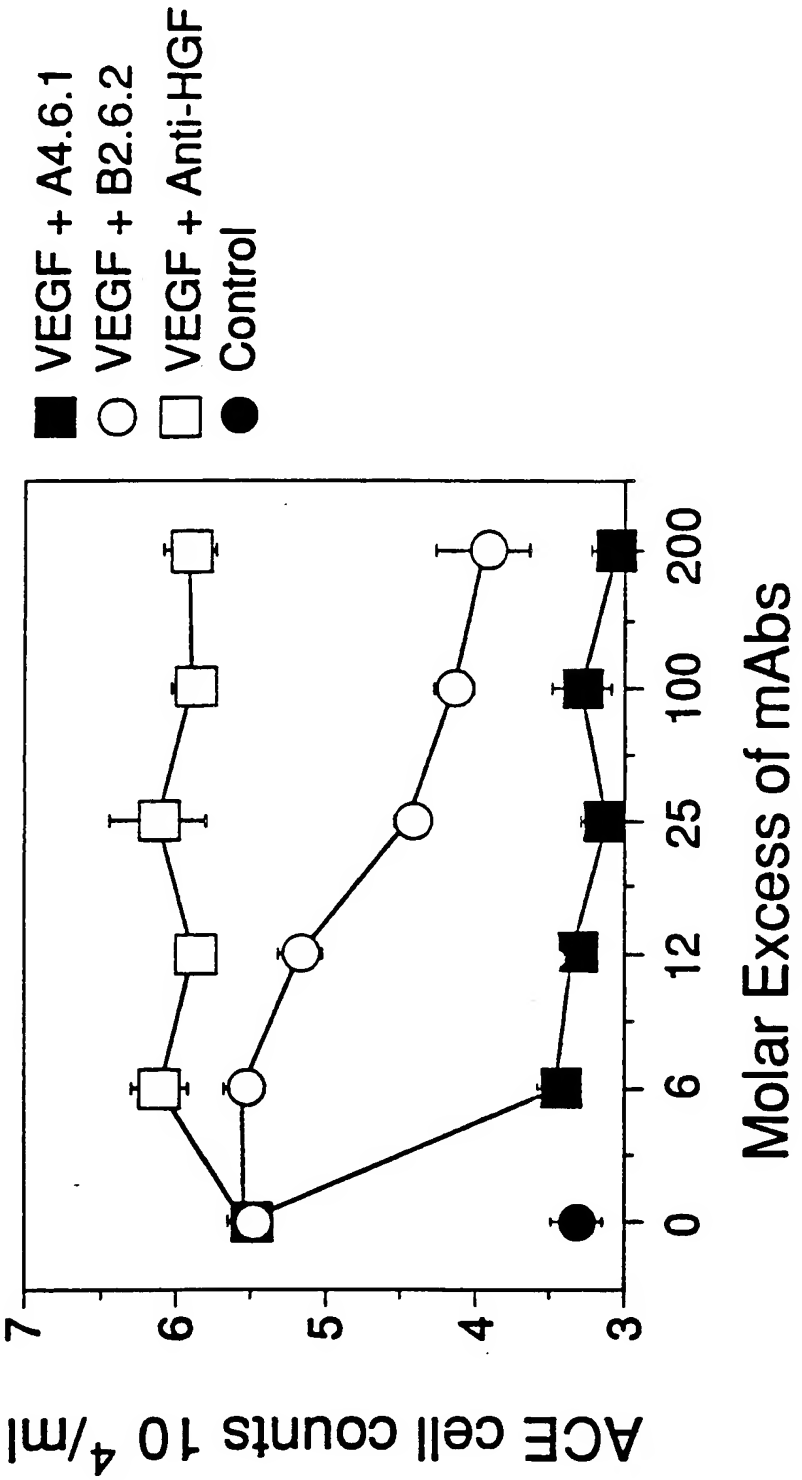

VEGF + A4.6.1
VEGF + B2.6.2
VEGF + Anti-HGF
Control
ACE cell counts 10 4/ml
7
6
5
4
3
0
6
12
25
100
200
Molar Excess of mAbs

FIGURE 2

FIGURE 3

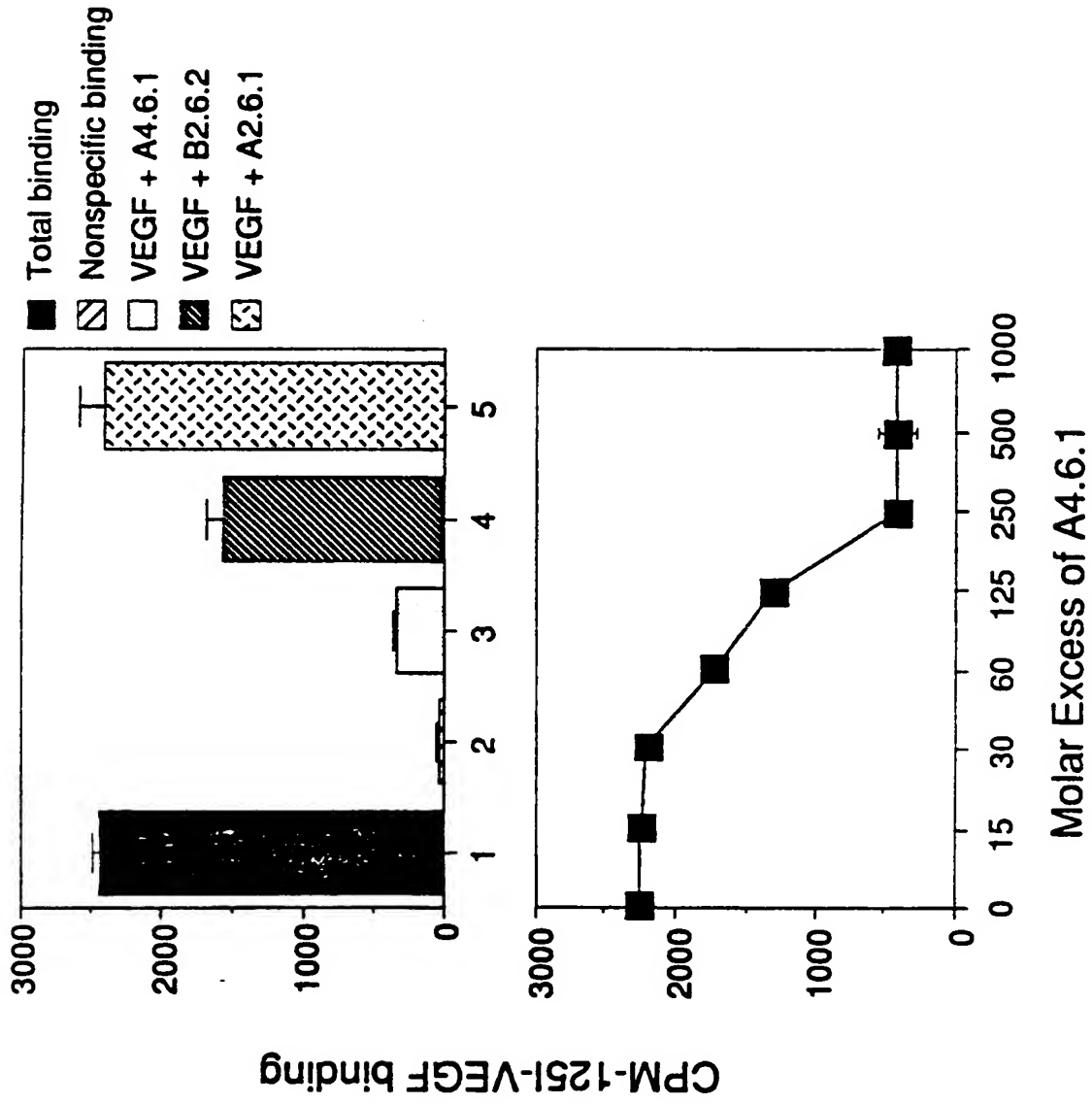

Total binding
Nonspecific binding
VEGF + A4.6.1
VEGF + B2.6.2
VEGF + A2.6.1
CPM-125I-VEGF binding
3000
2000
1000
0
1
2
3
4
5
0
15
30
60
125
250
500
1000
Molar Excess of A4.6.1

Figure 4

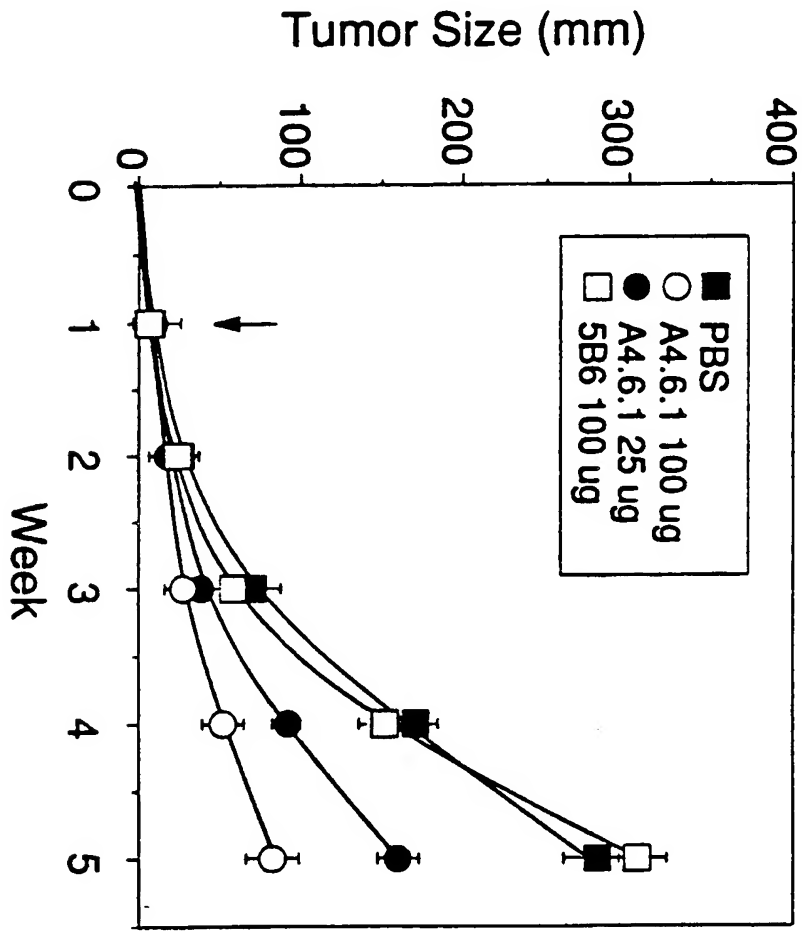

Tumor Size (mm)
0
100
200
300
400
Week
0
1
2
3
4
5
PBS
A4.6.1 100 ug
A4.6.1 25 ug
5B6 100 ug

Figure 5

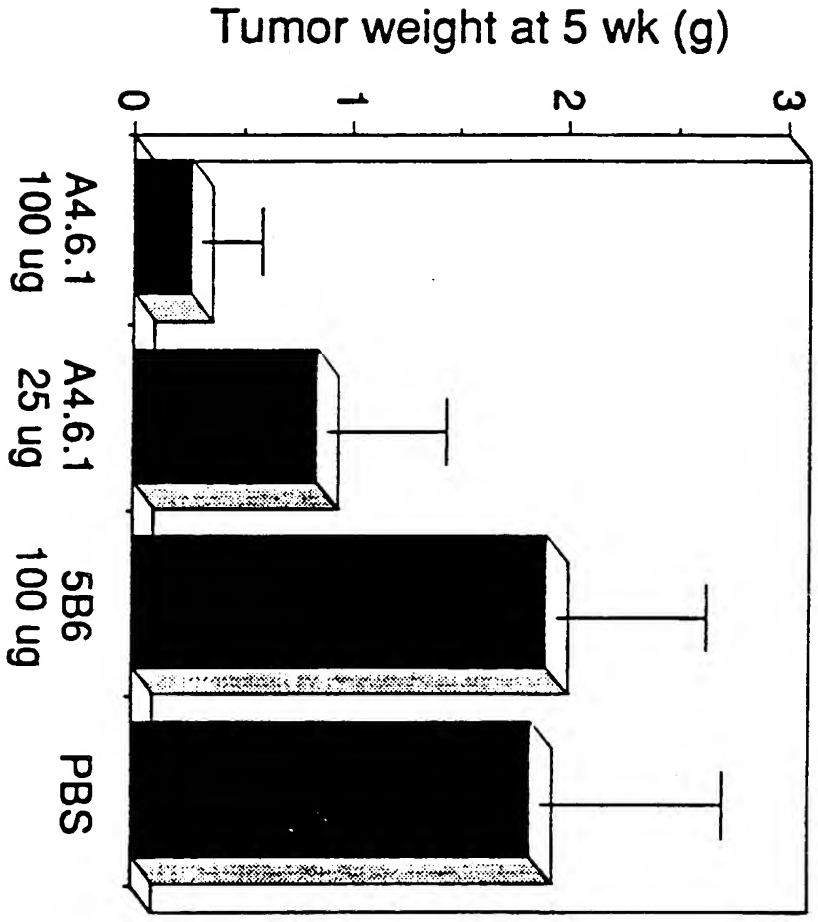


Figure 6

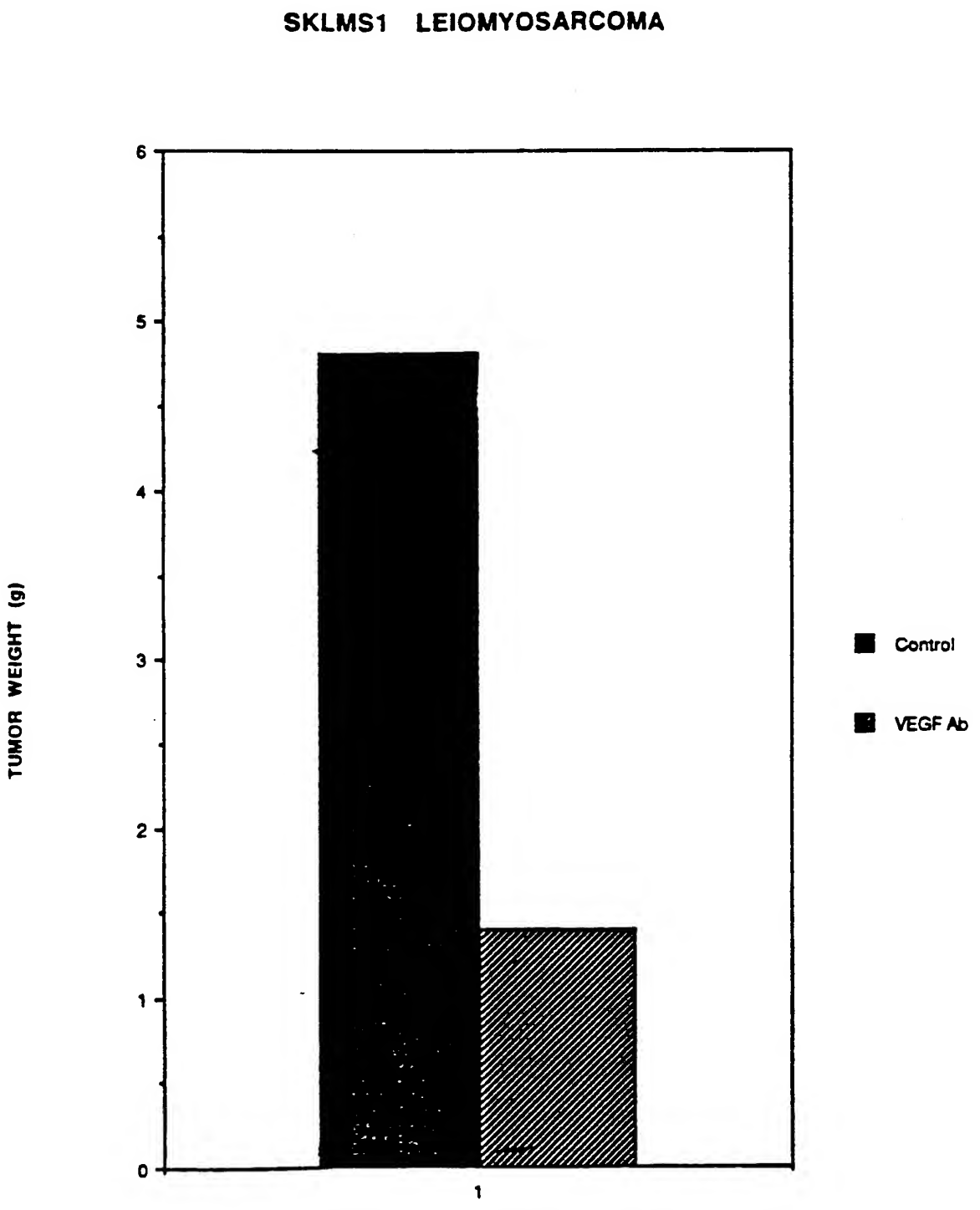
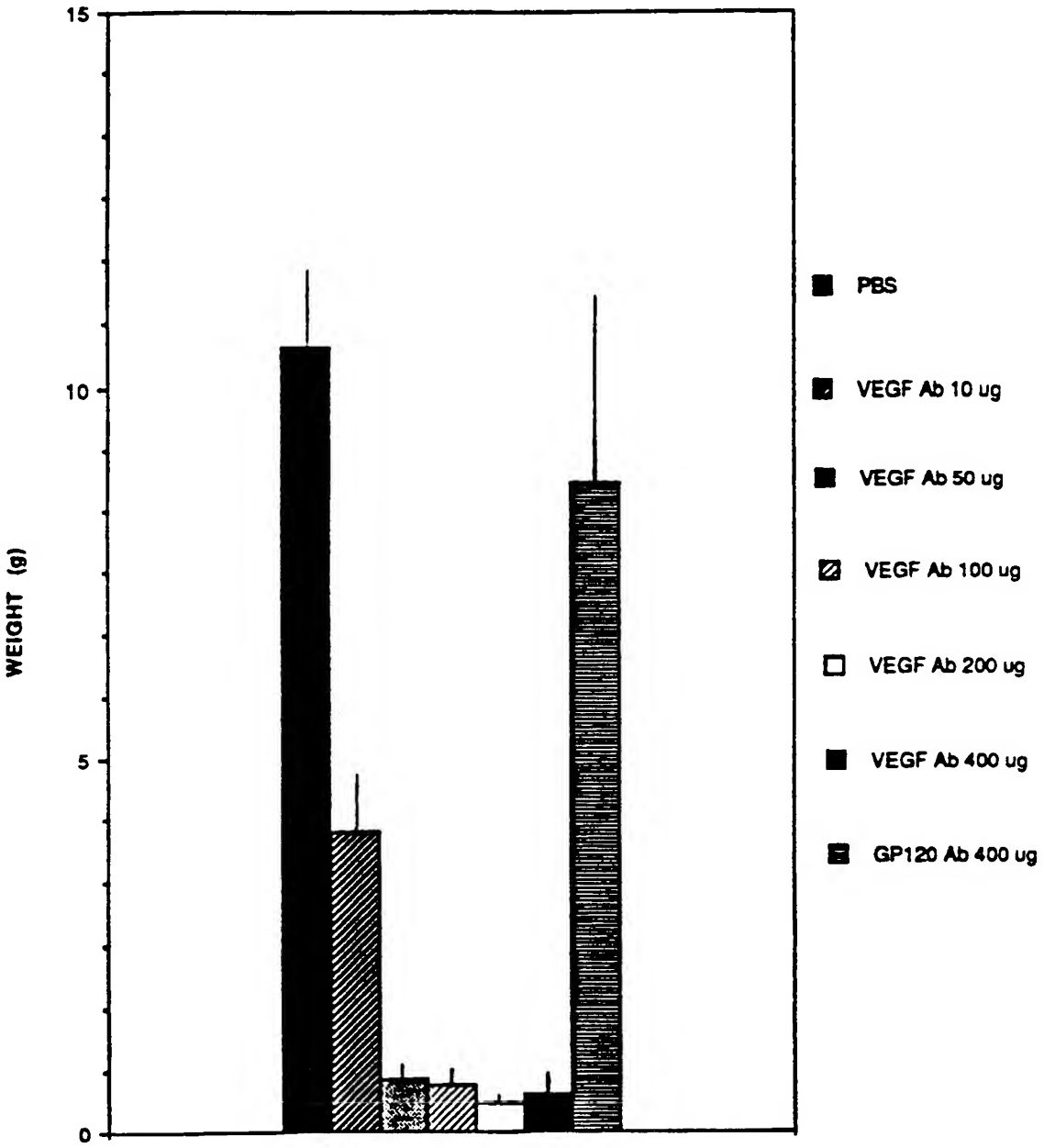

SKLMS1 LEIOMYOSARCOMA
TUMOR WEIGHT (g)
6
5
4
3
2
1
0
1
Control
VEGF Ab

Figure 7

A673 RHABDOMYOSARCOMA.
TUMOR WEIGHT FOUR WEEKS AFTER INJECTION



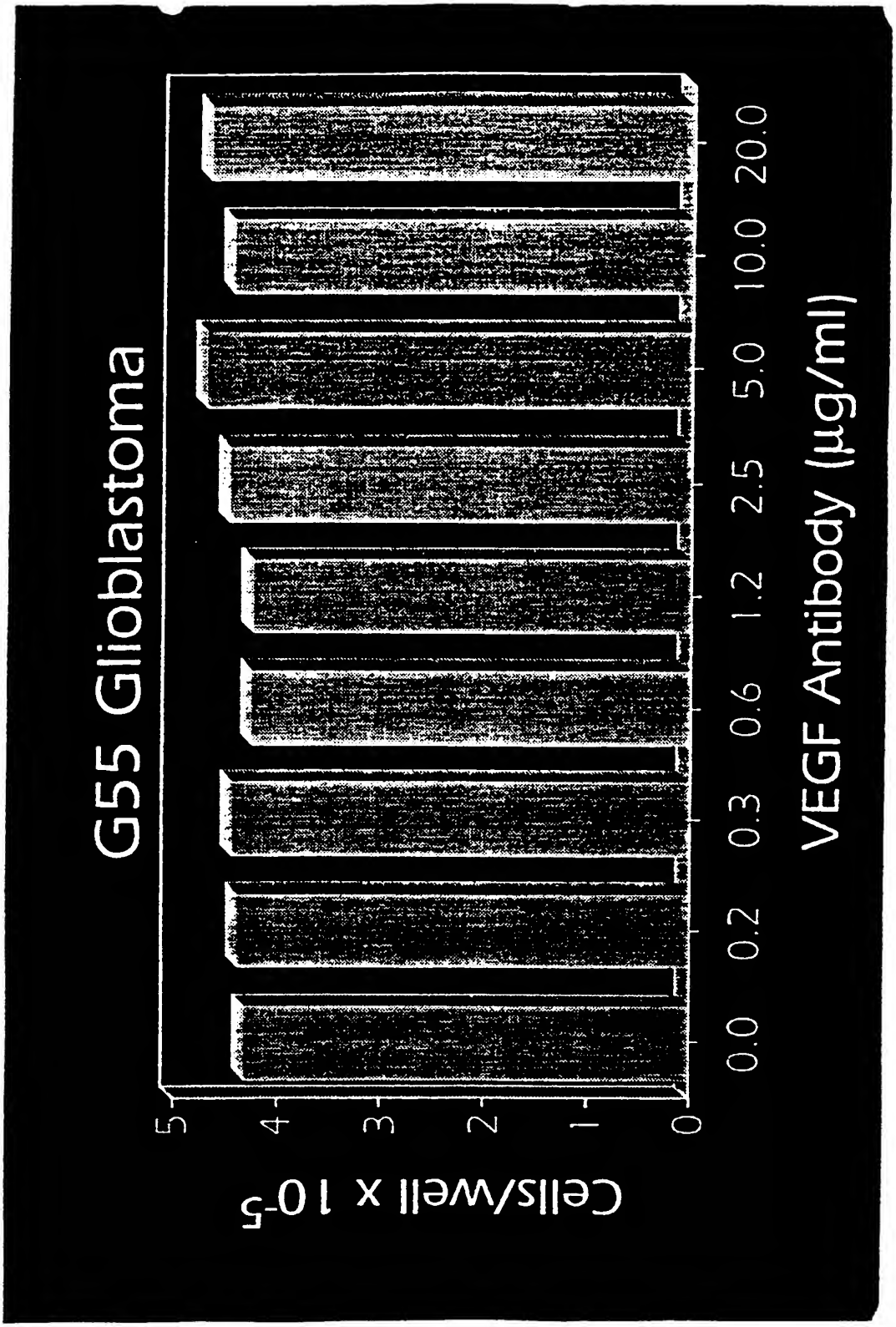

G55 Glioblastoma
Cells/well x 10-5
0
1
2
3
4
5
0.0
0.2
0.3
0.6
1.2
2.5
5.0
10.0
20.0
VEGF Antibody (µg/ml)

Figure 8

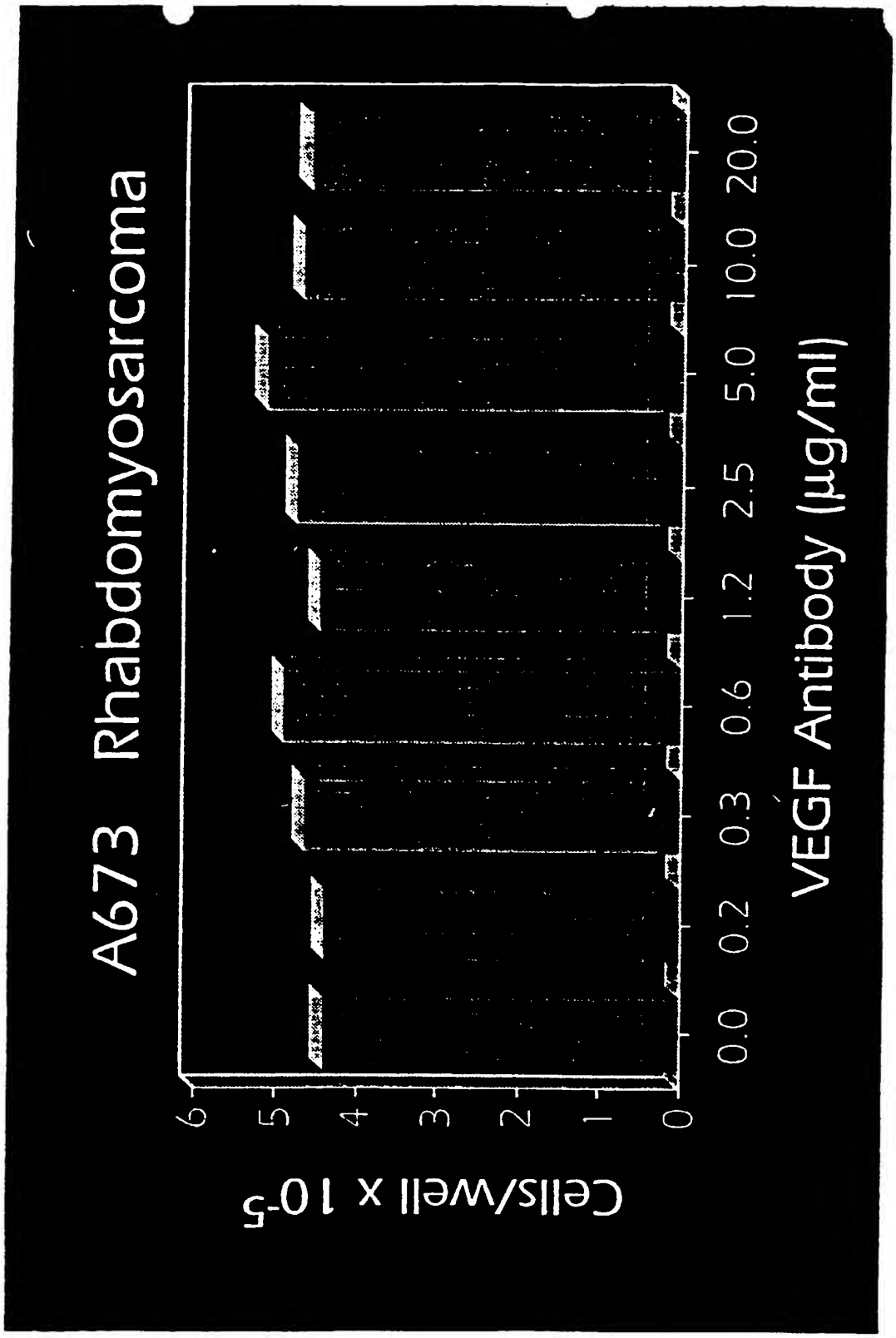

A673 Rhabdomyosarcoma
Cells/well x 10-5
0
1
2
3
4
5
6
0.0
0.2
0.3
0.6
1.2
2.5
5.0
10.0
20.0
VEGF Antibody (µg/ml)

Figure 9

Figure 10

Endothelial Chemotaxis (cell number)

Sample Type	Sample #	Assay Date	Syn. Fluid	Syn. Fluid + mAB VEGF	% Suppression
Rheumatoid Syn. Fluid	318	5.7.92	5.2±0.2	2.7±0.3	48
	150	5.7.92	7.0±0.3	2.8±0.4	60
	312	5.7.92	6.7±0.4	3.7±0.3	45
	264	5.7.92	6.2±0.4	3.1±0.3	50
	267	5.7.92	5.7±0.6	4.4±0.3	23
	202	5.22.92	10.0±0.5	3.4±0.6	66
	314	5.22.92	7.5±0.3	3.1±0.6	59
	237	5.22.92	6.1±0.5	2.2±0.3	64
	206	5.22.92	6.7±0.5	2.2±0.3	67
	317	5.22.92	5.2±0.3	2.5±0.6	52
Osteoarthritis Syn. Fluid	165	6.2.92	4.0±0.3	2.8±0.4	30
	211	6.2.92	3.4±0.5	3.0±0.2	11.7
	195	6.2.92	3.5±0.2	3.3±0.3	5.7
	122	6.2.92	3.7±0.3	3.2±0.4	13.5
	16	6.2.92	4.1±0.3	3.8±0.5	7.3

Mean % Suppression for RA Fluids 53.4±4.2
Mean % Suppression for OA Fluids 13.6±3.9
Synovial fluids were diluted 1:50.

Controls:

6.2.92	PBS	3.3±0.30
	bFGF 1µg/ml	5.7±0.38
5.22.92	PBS	1.2±0.38
	bFGF 1µg/ml	7.8±0.31
5.2.92	PBS	1.3±0.18
	bFGF 1µg/ml	9.0±0.41

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/09218

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K15/00; C12P21/08; A61K39/395; A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; C12P ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY vol. 264, no. 33, 25 November 1989, WASHINGTON DC, US pages 20017 - 20024 D. CONNOLLY ET AL. 'Human vascular permeability factor. Isolation from U937 cells.' see abstract see page 20021, left column, line 3 - right column, line 13 ---	1,3,5,6
A	JOURNAL OF CELLULAR BIOCHEMISTRY vol. SUPPL, no. 15F, 1991, NEW YORK, US page 251 B. LI ET AL. 'Monoclonal antibodies to recombinant human vascular endothelial growth factor (rHuVEGF).' see abstract CF 417 -----	1

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

1

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 JUNE 1993	08 -07- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NOOIJ F.J.M.

Form PCT/ISA/210 (second sheet) (January 1985)

INTERNATIONAL SEARCH REPORT

Inte onal application No.
PCT/US92/09218

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark :Although claims 27-30 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort jusufying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.